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An In-depth Characterisation of Neonatal Seizures by Early Continuous Video-EEG Analysis

Evonne Low

Submitted in January 2016



A thesis submitted to the National University of Ireland, Cork

in conformity with the requirements for

the Degree of Ph. D.

This research was conducted at the Department of Neonatology,
Cork University Maternity Hospital, Neonatal Brain Research Group,
Irish Centre for Fetal and Neonatal Translational Research,
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Declaration

This thesis is my own work. It has not been submitted for another degree, either at the National University of Ireland, Cork or elsewhere.

Evonne Low

Abstract

Introduction To date, neonatal seizures remain a challenge to many researchers; both in the clinical and non-clinical fields worldwide. More compelling laboratory and clinical evidences are showing that seizures are harmful to the neonatal developing brain and that poor long-term neurodevelopmental outcome has been associated with neonatal seizures. The clinical management of neonatal seizures can be optimized through more reliable and accurate method of seizure detection, if more effective neuroprotective strategies are available in treating these seizure and to delineate how these treatment strategies affects the seizure burden in the neonatal developing brain.

Aims Information on the characteristics of seizure burden from current population of neonates in the neonatal intensive care unit needs to be investigated. This would include cooled HIE neonates with hypoxic-ischaemic encephalopathy (HIE) (as therapeutic hypothermia has become the standard of care in clinical practice for term neonates with HIE) and neonates with stroke, which had been previously known to be the second most common identifiable cause of seizures in term neonates. It will also be more informative to assess the response of seizure burden inclusively in neonates with seizures due to other aetiologies when our current treatment strategy of anti-seizure medication is applied.

The following 4 specific studies were undertaken:

Study 1: Comparison on the seizure profile between non-cooled versus cooled neonates with HIE (the Cooling study).

Study 2: The characteristics of seizure profile in neonates with stroke (the Stroke study), based on detailed characteristics of EEG seizures analysis (the Stroke study),

Study 3: The response of seizure burden to treatment with anti-seizure medication in term neonates (Phenobarbitone study).

Study 4: In term neonates, electroclinical dissociation of seizures (ECD) has been speculated to have a high occurrence. However, it has not been quantified using multichannel EEG and in a cohort of term neonates with multiple aetiologies which are presented in the neonatal intensive care unit. This study aimed to determine the degree of ECD occurrence in the current population of term neonates with seizures.

Methods The multichannel video-EEG was used in this research study as the gold standard to detect seizures, thus allowing an accurate quantification of seizure burden to be ascertained. Neonates more than 37 weeks gestation who were at high risk of developing seizures, were enrolled for EEG monitoring as soon as possible after delivery. Neonates were recruited from the neonatal intensive care units at Cork University Maternity Hospital (CUMH), Ireland and from Elizabeth Garrett Anderson Wing, University College London Hospital, London (UCLH), United Kingdom, between 5th January 2009 and 1st October 2011. A historical cohort of non-cooled term neonates recruited from 1st June 2003 to 31st December 2006, was included for comparison with neonates who were cooled.

The entire EEG recording for each neonate was independently reviewed and annotated by at least 1 experienced neurophysiologist. All analyses were done using the PASW Statistics 17.0, 18.0, 20.0 and SAS 9.3. Data were treated as non-parametric and expressed in medians and interquartile ranges (IQR). The interrater agreement between 2 electroencephalographers was assessed using a Cohen's Kappa (κ) statistic. For comparisons between the two groups (non-cooled and cooled), the Mann-Whitney test was used for continuous variables and the χ^2 test or Fisher's exact test (in the case of small expected counts) was used for categorical variables. For paired comparisons, the Wilcoxon signed-rank test was used. For comparisons between groups, group was included as a fixed effect in the linear mixed model. Results based on linear mixed models were presented as mean [95% confidence interval]. A p value <0.05 was deemed as significant.

Results In the Cooling study, thirty seven neonates were identified to have electrographic seizures; of these, 31 had recordings that were suitable for analysis (16 non-cooled, 15 cooled). Compared with non-cooled neonates, earlier [age: 6 (3-9) vs 15 (5-20) hours] and longer [88 (75-101) vs 55 (41-60) hours] EEG monitoring were undertaken in cooled neonates. Despite this increased opportunity to capture seizures in cooled neonates, the recorded seizure burden in the cooled group was significantly lower than in the non-cooled group [60 (39-224) vs 203 (141-406) minutes; $p=0.027$]. Further exploratory analysis showed that the recorded seizure burden was only significantly reduced in cooled neonates with moderate HIE [49 (26-89) vs 162 (97-262) minutes; $p=0.020$] when compared with severe HIE.

In the Stroke study, nine neonates with perinatal arterial ischaemic stroke seizures and EEG monitoring were identified. While EEG continuity was present in all cases, the background pattern showed suppression over the infarcted side; this was quite marked (>50% amplitude reduction) when the lesion was large (>66% of one hemisphere). Characteristic unilateral bursts of theta activity with sharp or spike waves intermixed were seen in all cases. Sleep cycling was generally present but was more disturbed over the infarcted side. Seizures demonstrated a characteristic pattern; focal sharp waves/spike-polyspikes were seen at frequency of 1 to 2 Hz and phase reversal over the central region was common. There were more electrographic-only than electroclinical seizures (78 vs 22%).

In the Phenobarbitone study, of the thirty-three neonates treated with phenobarbitone, 19 were treated concurrently with electrographic seizures. The seizure burden was significantly reduced within 1 hour of phenobarbitone administration [mean (95% confidence interval): -14 (-20 to -8) minutes/hour; $p<0.001$]. Seizures abated in 3 neonates while in 16 neonates, I have found that seizures returned to levels not significantly different to pre-treatment levels within 4 hours of first phenobarbitone administration ($p=0.064$), before seizures returned more aggressively requiring further use of second-line anti-seizure medication. Compared with 10 mg/kg doses, a subgroup analysis revealed that only phenobarbitone doses at 20 mg/kg resulted in a significant reduction in seizure burden ($p=0.004$).

In the Electroclinical dissociation study, the ECD index in the cooled neonates with HIE, in non-cooled neonates with HIE, neonates with focal stroke and in neonates with other diagnoses were 88%, 94%, 64% and 75% respectively.

Conclusions Prolonged and continuous multichannel video-EEG monitoring is advocated for adequate seizure surveillance. Compared to neonates with severe HIE, a decreased seizure burden was noted in neonates with moderate HIE who received therapeutic hypothermia. This finding may explain some of the therapeutic benefits of hypothermia seen in term neonates with moderate HIE. EEG monitoring aids in early identification of neonates with neonates and allows us to delineate the true extent of electroclinical dissociation of seizures after treatment has been instigated. Electrographic seizures in the absence of clinical seizures can recur with a higher incidence, particularly after instigation of treatment and cooling of neonates with HIE compared to neonates who had seizures stemming from other diagnoses. Further research on the precise mechanistic action of neuroprotective strategies, including phenobarbitone in the human developing neonatal brain is required if our clinical incentive is to abolish seizures in the developing neonatal brain. A change to our current treatment strategy is required, as we continue aiming to strive for more effective seizure control, anchored with the use the use of prolonged and continuous multichannel EEG as the surveillance tool.

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Abbreviations

| | |
|-------|--|
| aEEG | Amplitude-integrated EEG |
| ASM | Anti-seizure medication |
| AMPA | Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate |
| ATP | Adenosine triphosphate |
| CFM | Cerebral function monitor |
| CI | Confidence interval |
| CUMH | Cork University Maternity Hospital |
| ECD | Electroclinical dissociation |
| ECG | Electrocardiogram |
| ECS | Electroclinical seizures |
| EEG | Electroencephalogram |
| EOS | Electrographic-only seizures |
| GABA | Gamma-aminobutyric acid |
| HIE | Hypoxic-ischaemic encephalopathy |
| ISB | Instantaneous seizure burden |
| IQR | Interquartile ranges |
| KCC2 | Potassium-chloride co-transporter isoform 2 |
| LMCA | Left middle cerebral artery |
| LPCA | Left posterior cerebral artery |
| MCA | Middle cerebral artery |
| MRI | Magnetic resonance imaging |
| NASDA | Neonatal automated seizure detection algorithm |
| NICU | Neonatal intensive care unit |
| NKCC1 | Sodium-potassium-chloride co-transporter isoform 1 |
| NMDA | N-methyl-D-aspartate |
| PAIS | Perinatal arterial ischaemic stroke |
| PB | Phenobarbitone |
| RMCA | Right middle cerebral artery |
| UCC | University College Cork |
| UCLH | University College London Hospitals |

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Section 1

Introduction to Neonatal Seizures

Chapter 1

Introduction

1.1 Background of this research study

Seizures in sick neonates are generally associated with severe neurological consequences (Volpe JJ, 2008). There is accumulating and compelling evidence showing that seizures are harmful to the developing neonatal brain (Ben-Ari and Holmes, 2006; Friedman and Hu, 2014; Payne et al., 2014; Shah et al., 2014; Thibeault-Eybalin et al., 2009). The incidence of seizures in the neonatal period (the first 28 days for term neonates and before 44 weeks of corrected gestational age for preterm neonates) remains higher than other age groups (Volpe JJ, 2008).

The incidence of neonatal seizures has been reported to be as high as 57.7 per 1000 neonates weighing under 1500 grams and 2.8 per 1000 neonates between 2500 grams and 3999 grams (Kohelet et al., 2004). Evidently, the reported incidence rate of neonatal seizures differs considerably according to geographical area with varying clinical practices, gestational ages, birthweights, era when the study was undertaken and the definition used for neonatal seizures. Principally, it is also influenced by methods employed in detecting seizures in neonates. Accurate seizure detection is crucial if the ultimate aim is to treat seizures which may be harmful in the neonate in order to prevent dire long-term neurodevelopmental consequences.

Clinical evaluation is an insensitive method of identifying neonatal seizures as electrographic seizures will not always be detectable by clinical inspection. Seizure events, which have no obvious clinical manifestations have been referred to as 'occult', 'subclinical' or 'silent' seizures; the vast majority of neonatal seizures are of this nature (Malone et al., 2009; Murray et al., 2008; Wusthoff et al., 2011; Yap et al., 2009). Yet, direct visual inspection is currently employed in virtually all units where electroencephalogram (EEG) monitoring is not available. Contrastingly in neonatal units where EEG is available, the amplitude-integrated EEG (aEEG) is widely preferred among neonatologists (Azzopardi, 2015; Boylan et al., 2010; Boylan et al., 2013; Toso et al., 2014).

However, the aEEG is not a reliable tool for detecting seizures, as it cannot detect seizures which are of short duration, low amplitude, that do not generalize (Rennie et al., 2004; Shellhaas et al., 2007; Stewart et al., 2010) and seizures originating from other regions not detected by the limited aEEG electrode placement. There is also concern in relation to the inter-rater agreement particularly among new and inexperienced aEEG users (Boylan et al., 2010; Boylan et al., 2015; Rennie et al., 2004). To date, prolonged and continuous

multichannel EEG monitoring remains the gold standard for detecting seizures in neonates (Azzopardi, 2015; Boylan et al., 2013; Boylan et al., 2015; Shellhaas, 2015). However, in neonatal units that do not have the availability of a continuous multichannel EEG monitoring, it is better to have an aEEG service rather than relying on clinical observation alone.

1.2 Aims and scope of this thesis

Seizures have been shown to be harmful to the developing neonatal brain leading to poor long-term neurodevelopmental outcomes (Ben-Ari and Holmes, 2006; Maartens et al., 2012; Scher, 2003; Schiering et al., 2014; Thibeault-Eybalin et al., 2009; van der Heide et al., 2012). However it remains controversial as to whether the severity of brain injury presenting as the aetiology of seizures, the degree of seizure burden itself or both contribute to the poor long-term neurodevelopmental outcome seen in this group of neonates.

The current strategy for anti-seizure medication usage may need to be revisited as some seizures may be treated inappropriately. Seizures are rarely treated under tight EEG control. Further in-depth information about the characteristics of seizures before, during and after treatment begins is required. This information can only be acquired from multichannel EEG: the gold standard for seizure detection in neonates.

A single seizure has been shown to have the ability to alter the homeostatic state of the developing brain with adverse consequences (Cornejo et al., 2007). Neonatal seizures themselves have been shown to cause further injury and exacerbate existing injury in the developing neonatal brain (Thibeault-Eybalin et al., 2009) by increasing the central nervous system metabolic demand above energy provision (Wasterlain et al., 2010). Recurrent seizures may be deleterious to the brain even without disturbances of ventilation or perfusion, and can cause the release of excitatory amino acids such as glutamate (Volpe JJ, 2008).

In clinical practice, prolonged seizures potentiate the risk of permanent brain injury and treatment becomes progressively more difficult if not instigated promptly after the onset of seizure (Boylan et al., 2004; Painter et al., 1999; Payne et al., 2014). Since seizures are harmful to the neonatal brain, we need better ways to prevent this harm by identifying seizures reliably and treating seizures effectively. My hypotheses are as follows:

Hypothesis 1: Therapeutic hypothermia reduces seizure burden in term neonates with hypoxic-ischaemic encephalopathy (HIE).

Hypothesis 2: There are characteristic features of electrographic seizures in neonates with stroke, hence potentially making the diagnosis earlier than other cranial imaging modality. In addition, in the absence of cooling, seizure burden in neonates with stroke may be higher than anticipated.

Hypothesis 3: Administered doses of phenobarbitone lower than 20 mg/kg are not as effective as at 20 mg/kg. The current treatment strategy clearly questions the effectiveness of phenobarbitone in terms of dosage and the timing of administration.

Hypothesis 4: There is a high incidence of electroclinical dissociation (ECD) of seizures in term neonates. A new and current cohort of neonates with seizures including cooled neonates is needed to confirm and quantify this, so as to determine the dissociation rate according to different seizure aetiologies.

In the sick neonate, as part of the first crucial steps in neonatal care, it is essential to characterize features of neonatal seizures detected by multichannel video-EEG in order to facilitate appropriate and effective treatment in the neonatal intensive care unit (NICU). Current information on the characteristics of seizures in term neonates based on the evidence from multichannel EEG is required to enhance our medical management of neonatal seizures in terms of diagnosis and treatment. We need to have a more effective treatment strategy using anti-seizure medication when clinically managing this vulnerable group of neonates. In time, this will ultimately lead to better and optimal management of neonatal seizures by neonatal teams as a stepping stone to achieve better long-term neurodevelopmental outcome in this group of neonates.

In this research study, I aimed to determine the characteristics of seizures in term neonates using prolonged continuous multichannel video-EEG recording. To achieve my research aims, primarily four studies were completed for this thesis:

Study 1: Characteristics of seizures in neonates with hypoxic-ischaemic encephalopathy: non-cooled versus cooled neonates (the Cooling study). To date, therapeutic hypothermia has become the standard of care for neonates with hypoxic-ischaemic encephalopathy in most tertiary neonatal units, hence it is imperative to examine the seizure characteristics in cooled neonates with hypoxic-ischaemic encephalopathy. In this study, I investigated this

by quantifying the effect of therapeutic hypothermia on recorded seizure burden obtained from continuous multichannel video-EEG monitoring.

Study 2: Characteristics of seizures in neonates with stroke (the Stroke study). Most studies have focused on seizures due to hypoxic-ischaemic encephalopathy and less is known about seizures caused by stroke, which remains the second most identifiable cause of seizures in term neonates. In this study, I describe the characteristic electrographic seizure burden and morphology of term neonates with stroke.

Study 3: Characteristics of seizures in neonates treated with phenobarbitone (the Phenobarbitone study). As part of the process in assessing the effectiveness of current anti-seizure medication treatment strategy, this study aimed to determine the effect of phenobarbitone on neonatal seizures specifically in relation to the degree of reduction in electrographic seizure burden in term neonates during continuous and prolonged multichannel video-EEG monitoring.

Study 4: Characteristics of electroclinical dissociation (ECD) of seizures in term neonates (the Electroclinical dissociation study). Electroclinical dissociation of seizures is believed to be a common phenomenon, but has rarely been quantified using multichannel EEG and in a cohort of term neonates who were either cooled or non-cooled with multiple aetiologies. This study aimed to determine the occurrence of this phenomenon in the current population of term neonates with seizures.

Section 1 of this thesis introduces the main aspects of neonatal seizures; it comprises of 4 chapters.

- An introduction to this research thesis is found in Chapter 1. It comprises the hypotheses and aims of this research thesis.
- It is vital to understand the pathophysiology of neonatal seizure as it forms the rationale for treatment of seizures; these are described in Chapter 2. Seizure treatment strategies, specifically using anti-seizure medication and/or therapeutic hypothermia are also discussed in this chapter.
- Chapter 3 specifically describes the clinical aspects of seizures and the various methods currently used for seizure detection in the NICU; the limitations posed by these methods are also highlighted.
- Seizure recognition using multichannel EEG and the definition of an electrographic seizure are also discussed in Chapter 4.

In Section 2,

- Chapter 5 describes the methodology used during the course of this research study. The study population, standard protocols, equipment, data collection, storage and analysis are described here.

Section 3 comprises the results and discussions of the 4 main studies pursued for this research study; they are categorized into 4 individual chapters.

- Chapter 6 describes the characteristics of seizures between 2 groups of neonates: the non-cooled and cooled neonates with hypoxic-ischaemic encephalopathy (the Cooling study).
- Chapter 7 describes the characteristics of seizures in neonates with stroke (the Stroke study).
- Chapter 8 describes the characteristics of seizures in neonates who have been treated with phenobarbitone (the Phenobarbitone study).
- Chapter 9 describes the degree of electroclinical dissociation of seizures in our current cohort of term neonates (the Electroclinical dissociation study).

In Section 4,

- Chapter 10 of this thesis summarizes the conclusive findings derived from this research study. Discussions on the implications and future research which can be undertaken from this research study are also included.

Section 5 lists the contributions which have been made to the literature from this thesis.

Section 6 contains the references used in this thesis while Section 7 contains the Appendices.

1.3 Publications from this thesis

1. **E Low** & GB Boylan. Neonatal seizures in hypoxic-ischaemic encephalopathy: the impact of therapeutic hypothermia. Seizures of Onset in the First Two Years of Life. Progress in Epileptic Disorders 2015. pg:88-91.
2. **E Low**, GB Boylan, SR Mathieson, DM Murray, I Korotchikova, NJ Stevenson, V Livingstone, JM Rennie. Cooling and seizure burden in term neonates: an observational study. Archives of Disease in Childhood. Fetal and Neonatal Ed. 2012;97(4):F267-F272.
3. **E Low**, SR Mathieson, NJ Stevenson, CA Ryan, V Livingstone, CO Bogue, JM Rennie, GB Boylan. Early Postnatal EEG Features of Perinatal Arterial Ischaemic Stroke with Seizures. PLoS One. 2014 Jul 22;9(7):e100973. doi:10.1371/journal.pone.0100973. eCollection 2014.
4. **E Low**, NJ Stevenson, SR Mathieson, V Livingstone, CA Ryan, JM Rennie, GB Boylan. Phenobarbitone Revisited: Short-term Effects on Electrographic Seizures in the Newborn. Neonatology 2016 (Article accepted for publication).
5. **E Low**, NJ Stevenson, SR Mathieson, V Livingstone, CA Ryan, JM Rennie, GB Boylan. Electroclinical Dissociation of Seizures in Term Neonates. Seizure 2015 (Short communication under review).
6. **E Low**, EM Dempsey, CA Ryan, JM. Rennie, GB Boylan. EEG suppression associated with apneic episodes in a neonate. Case Reports in Neurological Medicine, vol. 2012, Article ID 250801, pages, 2012.doi:10.1155/2012/250801.
7. BH Walsh, **E Low**, CO Bogue, DM Murray, GB Boylan. Early continuous video electroencephalography in neonatal stroke. Dev Med Child Neurol. 2011 Jan;53(1):89-92. doi:10.1111/j.1469-8749.2010.03837.x.Epub 2010 Nov 18.
8. SR Mathieson, NJ Stevenson, **E Low**, WP Marnane, JM Rennie, A Temko, G Lightbody, GB Boylan. Validation of an Automated Seizure Detection Algorithm for Term Neonates. Clin Neurophysiol. 2015 May 9. pii:S1388-2457(15)003168.doi:10.1016/j.clinph.2015.04.075. [Epub ahead of print].

Chapter 2

Pathophysiology of Neonatal Seizures and their Basis for Treatment

Introduction

In 1870, J. Hughlings Jackson described a seizure as an “excessive discharge of nerve tissue on muscle” (Jackson, 1890). He elaborated “this discharge occurs in all degrees, with all sorts of conditions of ill health, at all ages and under innumerable circumstances”. Epidemiological evidence has shown that the highest risk for seizures occurs in the first decade of life, explicitly during the neonatal period (Silverstein and Jensen, 2007). A seizure is a sign or symptom of an underlying diagnosis affecting the developing neonatal brain. Seizures are the most common and prominent clinical manifestation seen when neurological injury has occurred during the neonatal period (Volpe JJ, 2008).

2.1 What are neonatal seizures?

2.1.1 Generation of neonatal seizures

Regardless of the underlying pathology or aetiology leading to a seizure, the theory is that all seizures are due to a shift in cell energy (Gillam-Krakauer and Carter, 2012). In neonates, the main theory to this shift in cell energy has been hypothesized as a result from an imbalance of inhibitory and excitatory neurotransmitters and from failure of the adenosine triphosphate (ATP)–dependent sodium-potassium (sodium-potassium) pump.

Fundamentally, the balance between excitation and inhibition determines whether a seizure occurs or not. It is the excitatory component which accounts for the generation of seizures. The developmental expression of receptors for inhibitory and excitatory neurotransmitters is age-dependent (Khazipov et al., 2004; Ritter et al., 2001); in that the immature brain of the human neonate differs considerably from the mature brain of the adult in the development and propagation of seizures. As a result of this, in response to an insult or injury to the brain, the developing neonatal brain is more susceptible to developing seizures than in the adult brain; and several mechanisms have been hypothesized to explain this (Jensen, 2009a; Jensen, 2009b). The main basic reasons for the hyperexcitable nature of the immature brain have been thought to be due to the enhanced excitatory neurotransmission, decreased expression of inhibitory mechanisms, developmental expression of neuronal ion channels, and age-dependent modulation of neuro-peptides.

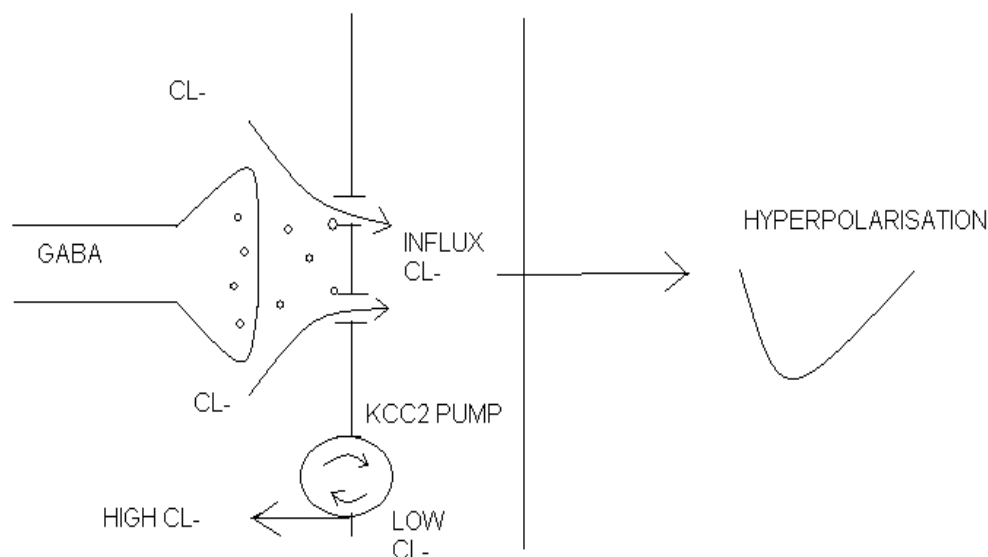
Enhanced excitability of the neonatal brain caused by excitatory nature of gamma-aminobutyric acid (GABA) neurotransmitter

The human model for seizure generation often describes two main neurotransmitters: glutamate and gamma-aminobutyric acid (GABA). In the adult brain, glutamate is the primary excitatory neurotransmitter and GABA is the principal inhibitory neurotransmitter (Cherubini and Ben-Ari, 2011); the latter prevents the spread of excitatory activity. However in the developing neonatal brain, controversy still remains as to whether GABA is inhibitory (Isaev *et al.*, 2007; Minlebaev *et al.*, 2007) or excitatory (Ben-Ari *et al.*, 2007; Ben-Ari *et al.*, 2008; Volpe JJ, 2008). A delayed in the development of inhibitory mechanisms has also been implicated (Moshe, 2000).

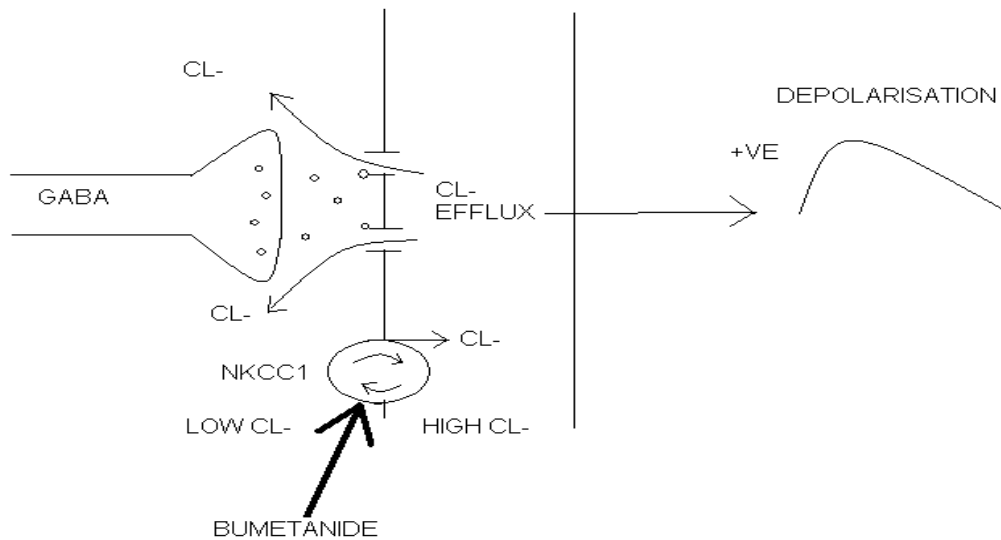
The more popular belief is that in the developing neonatal brain, GABA can provide a paradoxical excitatory drive due to the predominance of sodium-potassium-chloride co-transporter isoform 1 (NKCC1) which moves chloride into the cell and the lower expression of potassium-chloride co-transporter isoform 2 (KCC2) which moves chloride out of the cell (Volpe JJ, 2008) (figure 2.1). This results in a high intracellular chloride concentration in immature neurons leading to a depolarization state which renders the immature brain more susceptible to seizures (Demarque *et al.*, 2004); as seizures are known to be paroxysmal alterations in the neurological function as a result of excessive synchronous depolarization of neurons within the central nervous system.

Figure 2.1 Mode of action of sodium-potassium-chloride co-transporter isoform 1 (NKCC1) and potassium-chloride co-transporter isoform 2 (KCC2) in neonatal brain (adapted from Volpe JJ, 2008)

A. The KCC2 pumps chloride out of the post synaptic membrane creating a gradient across the membrane. Release of GABA at the synapse opens the chloride channel causing influx of chloride and hyperpolarisation.



B. NKCC1 (expressed in preterm less than term) pumps chloride into postsynaptic cell creating a high intracellular concentration. GABA released at the synapse opens chloride channels and led to chloride efflux and depolarisation.



There is considerable and growing evidence from animal models and human tissue studies showing that neurotransmitter receptors are highly developmentally regulated (Rakhade and Jensen, 2009; Sanchez and Jensen, 2001). The reason for GABA having an excitatory effect in the immature brain has been linked to the fact that the effects of GABA on chloride conductance change with age (Jensen, 2006; Jensen, 2009a). In the more mature cells, GABA has been shown to cause hyperpolarization because KCC2 is active. It has been hypothesized that the balance between excitatory versus inhibitory synapses is in the favour of excitation in the developing neonatal brain, so as to permit robust activity-dependent synaptic formation, plasticity and maturation (Rakhade and Jensen, 2009).

There have been concerns of neonatal seizures which are refractory to anti-seizure medications and of its severe consequences on long-term neurodevelopmental outcome (Boylan et al., 2015; Guthertz et al., 2014; Hellstrom-Westas et al., 2015). Developmental stage-specific factors and age-specific mechanisms have been hypothesized to influence mechanisms of seizure generation, responsiveness to anti-seizure medications (mainly with barbiturates and benzodiazepines), and the potential adverse impact on development of the central nervous system (Jensen, 2009a; Jensen, 2009b; Rakhade et al., 2011). The incomplete development of neurotransmitter systems in neonates have been linked to the lack of “target” receptors for anti-seizure medications (Puskarjov et al., 2014).

The higher expression of NKCC1 in the developing neonatal brain suggests that seizures may be resistant to treatment when a GABA agonist such as phenobarbitone is used (Volpe JJ, 2008). This is further supported by the fact that when bumetanide (a NKCC1 antagonist) is used in experimental models, it reduces intracellular chloride (Dzhala *et al.*, 2010) and had

shown efficacy against kainate-induced seizures in the immature brain (Dzhala et al., 2008); promoting the idea that GABA may be more excitatory during neonatal brain developmental (further discussion in section 2.4). However, an intricately designed European clinical trial has shown that bumetanide as an add-on to phenobarbitone does not improve seizure control in human term neonates with hypoxic-ischaemic encephalopathy, and that it leads to increase risk of hearing loss (Pressler et al., 2015). Future studies based on more convincing pathophysiological evidence are required to develop more effective anti-seizure medications to treat neonatal seizures.

Enhanced excitability of the neonatal brain caused by increased expression of glutamate receptors

An over-expression of certain glutamate receptor subtypes in both rodent and human developing cortex has been found to coincide with age and its increased susceptibility to developing seizures (Sanchez et al., 2001; Sanchez and Jensen, 2001; Talos et al., 2006). In neonates, it has been hypothesized that there is a relative excess of excitatory neurotransmitters and receptors, and that this increased in neuronal excitability has been linked to an increased receptor expression of glutamate receptors; namely the the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors and the N-methyl-D-aspartate (NMDA) receptors (Ben-Ari and Holmes, 2006; Jensen, 2006; Jensen, 2009a). In the immature brain, potassium tends to accumulate in the extracellular space, as a result of a decreased in sodium-potassium-ATPase activity, and immature enzyme systems.

NMDA receptors (NR) has an obligate known as the NR1 subunit which is also developmentally regulated (Jensen, 2009a). In the immature brain, the NR2 subunits are predominantly those of the NR2B subunit, which has a functional correlate that has a longer current decay time compared with the NR2A subunit, which is the form expressed in more mature neurons (Jiang et al., 2007). Other developmentally regulated functional subunits include the NR2C, NR2D, and NR3A subunits. Rodent studies show that these subunits increased in the first 2 postnatal weeks, exposing them to have a lower sensitivity to magnesium, the endogenous receptor channel blocker; and that these factors contribute to the increased neuronal excitability in the developing neonatal brain (Wong et al., 2002).

The NMDA receptor has been reported to be selectively activated during plasticity and learning, and that the AMPA subtype of glutamate receptor is thought to be involved in mostly the fast excitatory synaptic transmission. Due to the enhanced calcium permeability, the AMPA receptors in the immature brain, has been hypothesized to play an important role in contributing not only to excitability but also to activity-dependent signalling down-stream of the receptor (Talos et al., 2006). Both NMDA and AMPA receptors are expressed at levels

and with subunit composition that enhance excitability of neuronal networks around term gestational age in the human neonate.

Although present in the neonate, the AMPA receptors are not fully functional and the NMDA channels do not operate at normal membrane resting potentials; this is because of its voltage dependent blockage with magnesium. The effect of depolarization produced by GABA is sufficient to remove the voltage-dependent magnesium block from NMDA channels, thereby inducing a large influx of calcium into the immature neurons. Rodent studies show that AMPA receptor antagonists (for eg: topiramate and talampanel) appear to be potently effective against neonatal seizures, and more effective than NMDA receptor antagonists or GABA agonists (Aujla et al., 2009; Koh and Jensen, 2001; Liu et al., 2004).

Enhanced excitability of the neonatal brain caused by the configuration of neuronal ionic channels

Ionic channels also regulate the neuronal excitability in the developing neonatal brain (Jensen, 2009a). Mutations in the potassium channels, namely the KCNQ2 and KCNQ3 (which are associated with benign familial neonatal convulsions) interfere with the normal hyperpolarizing potassium current that prevents the repetitive firing of action potentials in the neurons (Yue and Yaari, 2004). Another related potassium channel subtype known as the hyperpolarization-activated cyclic nucleotide-gated (HCN or h) channels are important for maintenance of the resting membrane potential and dendritic excitability (Pape, 1996). The immature brain has a low expression of the HCN1 isoform, which reduces the dendritic excitability in the adult brain (Bender et al., 2001). The maturation of ionic channels can also contribute to the cumulative effect in the hyperexcitability state of the immature brain when occurring in combination with the differences in ligand-gated channels (Bender et al., 2001; Jensen, 2006; Jensen, 2009b).

Enhanced excitability of the neonatal brain caused by neuro-peptides

An interesting example of a neuropeptide is the corticotropin releasing hormone, which releases potent neuronal excitation (Clynen et al., 2014; Dobolyi et al., 2014; Wu et al., 2012). In the perinatal period, corticotropin releasing hormone and its receptors are expressed at higher levels than in later life, specifically in the first 2 postnatal weeks in the rat; corticotropin releasing hormone levels increase during stress, explaining perhaps why seizure activity in the immature brain may exacerbate subsequent seizure activity (Chu et al., 2013; Korosi et al., 2010). Neuropeptide modulation has the potential for future treatment of neonatal seizures (Clynen et al., 2014; Dobolyi et al., 2014).

2.1.2 Propagation of neonatal seizures, status epilepticus and epilepsy

Glial proliferation, neuronal migration, myelin deposition, establishment of complex axonal and dendritic communications are incomplete in the neonatal brain (Volpe JJ, 2008); this contributes to the difference in the propagation or spread of seizures when compared to the adult brain. The propagation of seizures in the immature developing neonatal brain is dependent on subcortical structures, particularly on the communications between the superficial and deep grey matter (Holmes and Ben-Ari, 2001; Peng et al., 2013). In the mature brain, the propagation of seizures relies on the communications at the level of the cortical grey matter of the cortex. The immaturity of the developing neonatal brain explains why electrical discharges are incompletely spread and tend to remain localized to one hemisphere in the neonatal brain.

If seizures are repetitive, a change in cerebral excitability could lead to status epilepticus (prolonged or recurrent seizures) (Lawrence and Inder, 2010) (further discussion in section 4.3). The Hebbian principle states that “neurons that fire together, wire together” (Hebb, 1967). This principle hypothesizes that early in development, spontaneous activities are generated based on the configuration of neuronal connections in the brain and are programmed necessary for function and survival. This supports the theory which hypothesized that poor long-term neurodevelopmental outcome of neonatal seizures is due to seizure-induced alterations in surviving networks of neurons, even following brief neonatal seizures (Ben-Ari and Holmes, 2006; Jensen, 2006; Jensen, 2009a). As a result, epilepsy or recurrent seizures beyond the neonatal period is a consequence of a disorder in the neuronal network which synchronously discharges.

Rapid increases in synaptic potency have been hypothesized to mimic long-term potentiation, and this activation may contribute to enhanced epileptogenesis (Rakhade et al., 2008). In the developing brain, the glutamate receptor-mediated molecular cascades have been thought to be associated with physiological synaptic plasticity which are over-activated by seizures (Cornejo et al., 2007; Rakhade et al., 2008). Many models reveal that neonatal seizures alter synaptic plasticity (Rakhade et al., 2011; Stafstrom et al., 2006), and alter the molecular signalling cascades (Raol et al., 2006; Sanchez et al., 2005).

In addition to glutamate receptors, inhibitory GABA_A receptors can also be affected by seizures in early life, resulting in long-term functional impairments. Following hypoxia-induced seizures in neonatal rat model, early and immediate functional decreases in inhibitory GABAergic synapses mediated by post-translational changes in GABA_A subunits are observed (Sanchez et al., 2005). There is evidence that some of these changes may be downstream of calcium permeable glutamate receptors and calcium signalling cascades, and

that early post-seizure treatment with glutamate receptor antagonists or phosphatase inhibitors may interrupt these pathological changes which may contribute to the adverse neurodevelopmental outcome and epilepsy (Rakhade et al., 2008; Sanchez et al., 2005).

Even less is known about how and why seizures end (Cross, 2014). Neuronal membranes, synapses, neurons and interneurons, subcortical structures moderating the balance between inhibition and excitation (Lado and Moshe, 2008), effects induced by neuromodulators (endocannabinoids, adenosine, neuropeptide Y) depletion of inhibitory neurotransmission (glutamate, GABA), failure of gap junction decoupling have been postulated as possible contributors as to why seizures end (Cross, 2014). It has also been hypothesized that seizures end when there is activation of inhibitory circuits in the neuronal network or changes in the ionic environment, such as a reduction in extracellular potassium (potassium currents activated by ion entry and loss of ionic gradients) or an elimination of intracellular calcium (Holmes and Ben-Ari, 2001). In animal models, seizures have been shown to end subsequently when there is depletion of energy substrates (Kovac et al., 2013; Wasterlain et al., 2010).

2.2 Risk factors for neonatal seizures

Although the neonatal brain is already at high risk of developing seizures compared to the adult brain, a subgroup of neonates who are at a higher risk of developing seizures can be identified through clinical history and examination. There are various risk factors identified for seizures (table 2.1), some of which often serve as inclusion criteria for EEG monitoring in neonates and therefore have been chosen for this research study (Chapter 5: Methodology).

| Table 2.1 Risk factors for neonatal seizures (adapted from Rennie JM et al., 2008) | |
|--|--|
| Abnormal cardiotopography in labour | Instrumental delivery |
| Depressed Apgar score (<5 at 5 minutes) | Emergency Caesarean section delivery during labour |
| Need for resuscitation at birth | Neonatal pyrexia |
| Low fetal scalp or cord pH (< 7.0) | Abnormal neonatal neurological behaviour |
| Prolonged rupture of membrane | Family history of neonatal seizures |
| Maternal pyrexia in labour and post-partum | Prematurity |
| Maternal drug abuse | Small for gestational age |

Risk factors based on pH and Apgar score for assessing hypoxic-ischaemia encephalopathy

According to a recently published national guideline in Ireland (Twomey A and Bowden A, 2011), therapeutic hypothermia should be considered for term neonates who present with clinical signs of moderate encephalopathy within the first 6 hours of age and who fulfill 1 of 4 of the inclusion criteria namely:

1. A need for positive pressure ventilation at 10 minutes of age.
2. An Apgar score less than or equal to 5 at 10 minutes of age.
3. pH of less 7.0 within the first hour of age.
4. A base deficit of more than or equal to 16 mmol/L within the first hour of age.


There is no clear diagnostic test for hypoxic-ischaemic encephalopathy (HIE), so sometimes it can still be very challenging and difficult to detect the sentinel of events leading to HIE.

Although the need for ventilation in an attempt to attenuate conditions relating to hypoxia is important, the inclusion criterion for positive pressure ventilation at the timeframe of 10 minutes of age is only arbitrary. In neonates with perinatal asphyxia, combined markers of illness such as Sarnat encephalopathy grade, Apgar score, intubation status, and pH had only a 25% positive predictive value and a 77% negative predictive value for seizure occurrence (Murray et al., 2006a)

Apgar score

Although the Sarnat score (Sarnat and Sarnat, 1976) and the Thompson score (Thompson et al., 1997) have been used to assess the early neurological condition of a neonate at different timepoints after delivery, the Apgar score remains the most common system used to assess the immediate condition of the neonate shortly after delivery. The Apgar score was originally designed by Virginia Apgar (table 2.2), an anaesthesiologist to assess the newborn's response to stress of labour and delivery. It is generally obtained every 5 minutes as in 1, 5, 10, 15 and even up to 20 minutes until the score is ≥ 7 .

The changes in the scores are generally relied on by clinicians to assess the efficacy of resuscitation. In some studies, the 10 minute Apgar score has been shown to be a prognostic value in encephalopathic neonates, even Apgar scores at 1 and 5 minutes had been shown to be associated with increased risk of later disability (Natarajan et al., 2013). However, the assesment of Apgar score is subjective at 1, 5 or 10 minutes and can vary significantly among nursing and medical personnel.

| Table 2.2 The Apgar scoring system (adapted from Apgar V, 1953) | | | | |
|--|----------------------------|-------------------------------------|--|--|
| Score | 0 | 1 | 2 | Dr. Virginia Apgar (1909-1974)  |
| Appearance (Skin colour) | Blue or pale | Blue at extremities, body pink | Extremities and body pink | |
| Pulse rate (heart rate) | Absent | <100 | >100 | |
| Grimace (Reflexes) | No response to stimulation | Grimace/ feeble cry when stimulated | Cry or pull away when stimulated | |
| Activity (muscle tone) | None | Some flexion | Flexed arms & legs that resist extension | |
| Respiratory effort (breathing effort) | Absent | Weak, irregular, gasping | Strong, lusty cry | |

pH

According to the American Committee of Obstetrics, the severity of hypoxic-ischaemic encephalopathy increases with an umbilical arterial base deficit of 12 to 16 mmol/L (ACOG, 2006). Moderate or severe hypoxic-ischaemic encephalopathy occurs in 10% of neonates who had this level of acidosis and that the rate increases to 40% in neonates who had umbilical arterial base deficit of greater than 16 mmol/L at birth. Although pH of less than 7.0 has been shown to be the most important umbilical blood gas variable for predicting early onset of neonatal seizures (Williams and Singh, 2002), cord pH (acidosis) are not sensitive, as congenital neonatal sepsis can also present with acidosis (Holcroft et al., 2004).

It has been shown that cord pH is not predictive of hypoxic-ischaemic encephalopathy (Murray et al., 2006b) or of neonatal seizures (Jonsson et al., 2014) but that brain alkalosis and high concentrations of cerebral lactate were associated with changes on cranial MRI consistent with severe brain injury (Uria-Avellanal and Robertson, 2014). Some asphyxiated neonates can appear clinically well shortly after delivery. They may not come to medical attention until they present with signs of encephalopathy or seizures at a later stage, by which time the presumed acidosis after delivery may have been compensated.

Clinical neurological assessment after resuscitation has high inter-observer variability. Accompanied with physical examination of the newborn (color, tone and cry), routine monitoring of vital signs using peripheral oxygenation and heart rate (as in Apgar score) do not reflect the immediate and ongoing states of the developing neonatal brain which may have already been compromised by variable degrees of hypoxia shortly after resuscitation. Depending entirely on physiological measurements may not be as reliable when assessing the severity of hypoxic-ischaemic encephalopathy.

More helpful to clinicians are information from cerebral physiological variables utilizing other feasible means of monitoring brain function in neonates such as using cranial ultrasound (static) or continuously (dynamic) by measuring ongoing cerebral oxygenation using near-infrared spectrometry and measuring ongoing cerebral electrical activity using EEG monitoring (Pichler et al., 2014; Tsuchida et al., 2013). During therapeutic hypothermia, monitoring cerebral function becomes even more crucial in providing invaluable cues to clinicians in terms of the managing the clinical aspects of neuroprotection, if the incentive is to optimize improvement in the long-term neurodevelopmental of neonates suspected of brain

injury and at risk of developing seizures (Glass et al., 2014; Tsuchida et al., 2013; Tsuchida, 2013).

Infection as a risk factor for seizures in the immature brain

Neonatal seizures can occur in the setting of inflammation resulting from an inter-current infection, hypoxic or ischaemic injury. Early microglial activation and inflammatory cytokine production has been shown to occur in the developing brain in both hypoxia or ischaemia (Huang et al., 2014; Zendedel et al., 2015). Microglia have been shown to be highly expressed in immature white matter in rodents and humans during cortical development (Billiards et al., 2006). In animal models experiencing an acute event of seizures, microglia activation has been demonstrated by morphologic changes and rapid production of pro-inflammatory cytokines (Abraham et al., 2012; Riazi et al., 2008).

2.3 Aetiology of neonatal seizures in term neonates

In term and preterm neonates, the aetiologies associated with seizures are different due to the dissimilar maturity of the cerebral structural organization between the 2 groups of neonates (table 2.3).

| Table 2.3 Causes of seizures in neonates (adapted from Rennie et al., 2008) | |
|---|--|
| Perinatal hypoxic-ischaemia Cerebral arterial infarction (perinatal arterial stroke) Cerebral venous sinus thrombosis Intracranial haemorrhage Subarachnoid haemorrhage Subdural haemorrhage Intraventricular haemorrhage Parenchymal (lobar) haemorrhage Thalamic haemorrhage Cerebellar haemorrhage Meningitis or encephalitis (bacterial, fungal or viral) Neonatal abstinence syndrome drug withdrawal from selective serotonin reuptake inhibitor, methadone drug intoxication Structural cerebral malformations | Metabolic causes Hypoglycaemia Hypo/ hypernatraemia Hypocalcaemia Hypomagnesaemia Hyperbilirubinaemia (kernicterus) Inborn errors of metabolism Pyridoxine dependency Biotinidase deficiency Glucose transporter type 1 deficiency Amino acids, organic acid disorders Syndromes Benign familial neonatal convulsions Benign non-familial neonatal convulsions Early infantile epileptic encephalopathy (Ohtahara syndrome) Neonatal myoclonic encephalopathy |

There are many causes of neonatal seizures in the term neonate, but only 2 main aetiologies are described in this chapter: hypoxic-ischaemic encephalopathy and stroke. This is because hypoxic-ischaemic encephalopathy remains the commonest cause of seizures in term neonates, while stroke is the second commonest identifiable cause of seizures in term neonates. The onset of seizures in term neonates with HIE characteristically ranged from 8 to 36 hours after birth (Pressler R.M, 2015). This appears to be similar to animal studies in which the EEG activity in lambs with an intrapartum insult is at first depressed, and then evolves to show electrographic seizure activity approximately 8 hours after birth

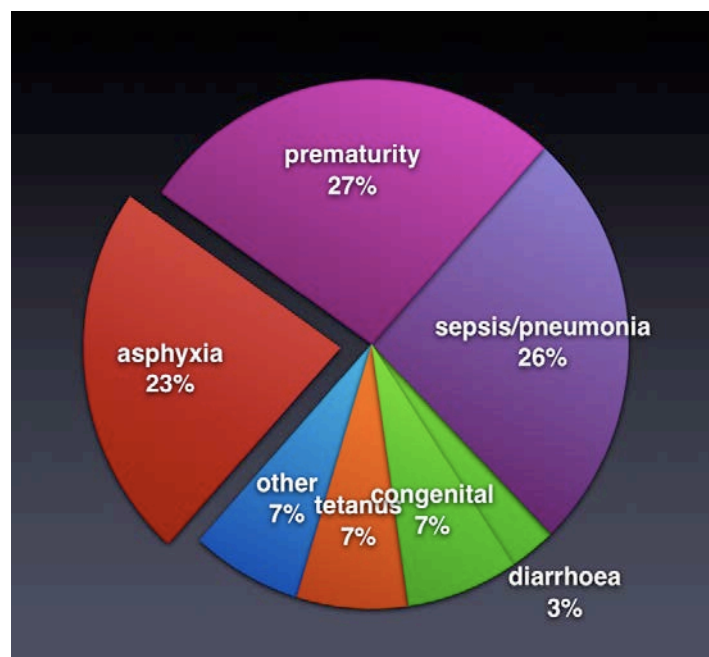
(Gunn et al., 1992). It was hypothesized that this would strongly suggest an antenatal insult which had occurred beyond 8 hours before delivery.

Early background EEG activity is a relatively reliable prognostic indicator for outcome (Pressler et al., 2001; Sampath et al., 2014). While therapeutic hypothermia has been shown to reduce seizures in HIE, it has not been advocated for neonates with stroke. To date, there is no existing intervention to reduce the seizure burden in neonates with stroke effectively; this may explain why seizures are still a common occurrence in neonates with stroke.

2.3.1 Hypoxic-ischaemic encephalopathy (HIE) and seizures

Birth asphyxia remains one of the leading causes of neonatal morbidity worldwide (Lawn et al., 2005a; Lawn et al., 2005b; Lawn et al., 2010) and perinatal hypoxia-ischaemia which affects approximately one to three per 1000 live term births, remains the most common cause of neonatal seizures, accounting for 40% of all cases (Volpe JJ, 2008) and is a major cause of long-term neuro-disability and death (Lawn et al., 2010; Marlow and Budge, 2005) (figure 2.2). In term neonates with hypoxic-ischaemic encephalopathy, seizures occur in approximately 50% of neonates with moderate and severe hypoxic-ischaemic encephalopathy (Levene et al., 1985; Sarnat and Sarnat, 1976), seizure onset of which is usually within the first 24 hours of life (Lynch et al., 2012). Studies completed before the widespread use of therapeutic hypothermia, show that traditional first and second-line anti-seizure medications to control seizures are often ineffective (Boylan et al., 2004; Painter et al., 1999).

Figure 2. 2 The millennium development goal is to reduce child deaths by two thirds by 2015 (adapted from Lawn et al., 2010)



Seizures have been shown to occur directly as a result of an asphyxial-induced brain injury. In experimental models of hypoxic-ischaemic encephalopathy, seizures occur either immediately after injury following an asphyxial insult, or in a delayed manner 6 to 12 hours after the initial insult when secondary energy failure leads to additional cell death (Scher et al., 2008). Gunn *et al.* found that if ischaemia lasted 30 minutes or longer, a stereotypic sequence of depressed EEG activity followed by a low frequency epileptiform activity was observed (Gunn et al., 1992).

The combination of hypoxia and seizures produces more profound changes in the brain rather than either factor alone (Wirrell et al., 2001). In neonatal animal studies, seizures add to hypoxic-ischaemic injury; the same may be true in the developing human neonatal brain (Miller et al., 2002; Wirrell et al., 2001). These seizures are often prolonged, frequent and status epilepticus from hypoxic-ischaemic encephalopathy is not rare. Histological findings in the hippocampus of 16 deceased and asphyxiated term neonates showed that there were alterations in the blood-brain barrier, increased activation of the microglia and greater expression of the inflammatory markers (namely interleukin 1 β and complement 1q) in neonates with seizures when compared with cases which had no seizures; this contributes further evidence that seizures lead to secondary brain injury (Schiering et al., 2014).

Following an extensive hypoxic-ischaemia insult in piglets at term equivalent age to human neonates, Bjorkman *et al.* demonstrated that seizures were associated with increased severity of brain injury (Bjorkman et al., 2010). Irrespective of the clinical manifestation of seizures, they showed that seizure activity was associated with a significant degree of brain injury as determined by histology, magnetic resonance imaging (MRI) and ¹H-magnetic resonance spectroscopy. The overlapping effects of brain injury from specific aetiologies versus seizure-induced brain injury *per se*, make it difficult to differentiate pre-existing brain lesions from direct and injurious effects of seizures themselves (Thibeault-Eybalin et al., 2009).

The severity of seizures in neonates with perinatal asphyxia has been shown to be independently associated with brain injury and adverse outcome (Garfinkle and Shevell, 2011; Miller et al., 2002). In response to hypoxemia-ischaemia, the preterm brain is most vulnerable in the white matter, whereas a term neonate has gray matter susceptibility (Back et al., 2007; Jensen, 2006). In a recent study, the

presence of neonatal status epilepticus was independently associated with epilepsy later on in life (Glass et al., 2011a). All of the children with epilepsy had injury noted on neonatal cranial MRI, with the majority who had injury demonstrated in the basal ganglia and thalamus.

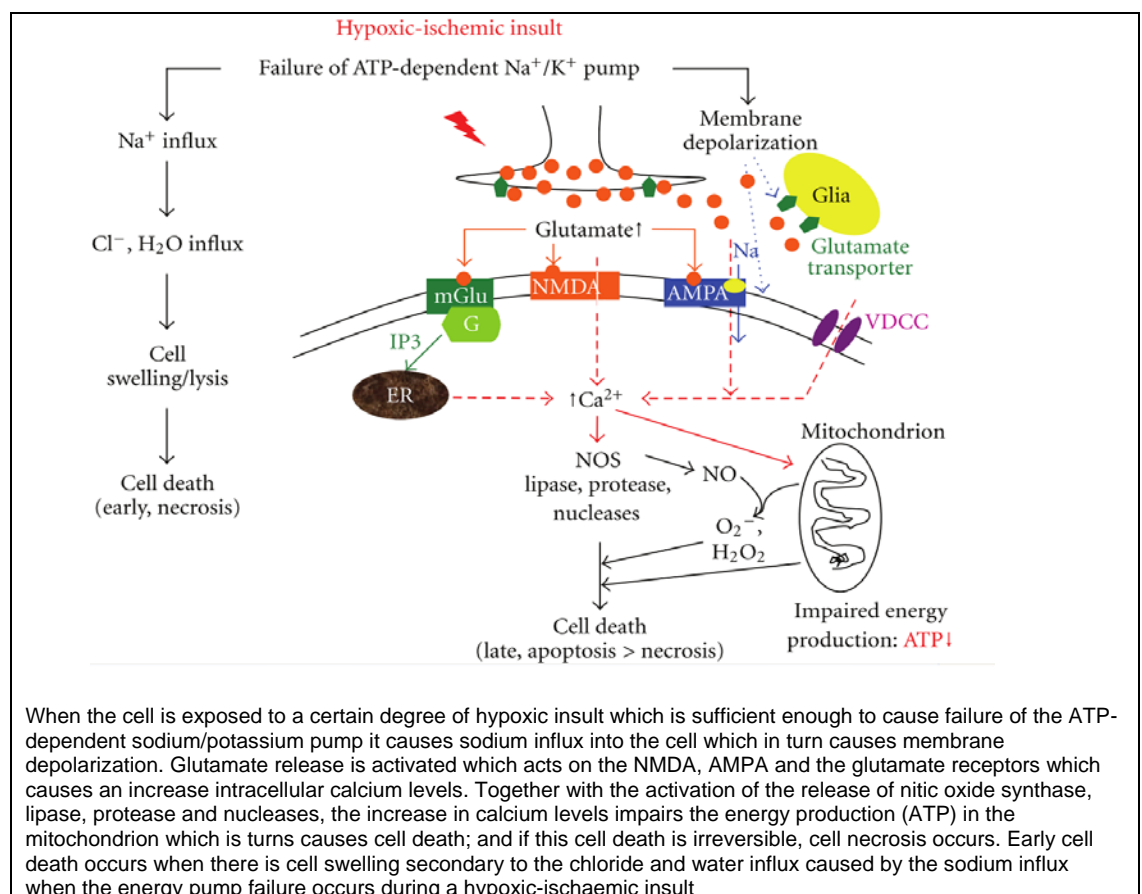
While examining the temporal distribution of seizures in neonates that did not receive therapeutic hypothermia, Lynch *et al.* found that seizures generally have a short period of high electrographic seizure burden followed by a longer period of low seizure burden, thereby resulting in an accumulation of seizures near the time of seizure onset (a positive skew) (Lynch et al., 2012). Seizures in human neonates with hypoxic-ischaemic encephalopathy may exacerbate the initial hypoxia-ischaemic injury and require treatment (Ancora et al., 2010; Glass et al., 2009; Miller et al., 2002; Shah et al., 2014) and there are some that would advocate for early seizure control (Boylan et al., 2004; DeLorenzo et al., 1999; Painter et al., 1999; Payne et al., 2014). However, this is very difficult to optimize without continuous video-EEG monitoring. The effects of cooling on seizures are discussed later in section 2.5 of this chapter.

The timing of primary and secondary phase of brain injury in hypoxic-ischaemic encephalopathy

The pre-existing state of the neonatal brain such as the degree of maturity (Dennis et al., 2013) and the frequency of repeated insults determines the severity of brain injury (Mallard et al., 1995). During the normal course of brain development, some neurons die (Gluckman and Williams, 1992), therefore many neurons do not only die during the asphyxial insult itself. However during hypoxia-ischaemia, the insult initiates cascades of processes that operate over a considerable time after the event (Gluckman and Williams, 1992), leading to a significant level of neuronal death (Lai and Yang, 2011) (figure 2.3).

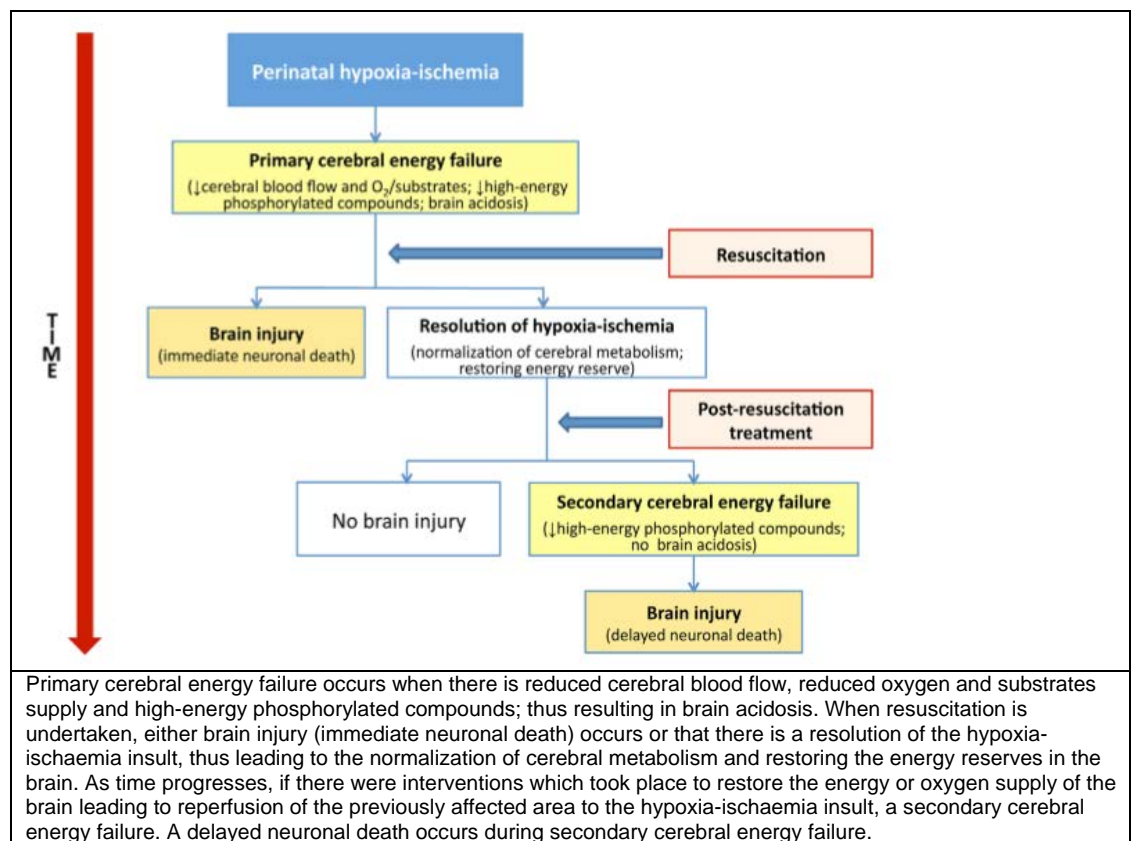
Figure 2.3 Cell death pathways in HIE (Lai and Yang, 2011)
Copyright © 2011 Lai MC and Yang SN. Perinatal Hypoxic-Ischemic Encephalopathy. J Biomed Biotechnol. 2011; 2011: 609813. Published online 2010 Dec 13. doi: 10.1155/2011/609813. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. <http://creativecommons.org/licenses/by/3.0/>

A. At the molecular level



Perinatal brain injury as a result from hypoxic-ischaemic encephalopathy in the term neonate is thought to be due primarily to a varying degrees of hypoxia and ischaemia (Ferriero, 2004; Johnston et al., 2011; Northington et al., 2011). Hypoxia describes the process that results from a lack of tissue oxygenation and perfusion (Evans DJ et al., 1999). The tissue undergoes a fall in cellular energy levels and an accumulation of carbon dioxide and lactic acidosis occurs through anaerobic metabolism. In animal models of perinatal hypoxia-ischaemia, 2 phases based on cerebral energy state have been commonly described: the primary and the secondary phase of energy failure (Antonucci R et al., 2014; Lorek et al., 1994) (figure 2.4).

Figure 2.4 Schematic diagram depicting the primary and secondary phases of energy failure following neonatal hypoxic-ischaemic encephalopathy (Antonucci R et al., 2014)
Copyright © 2014 Antonucci R, Porcella A, Pilloni MD. Perinatal asphyxia in the term newborn. J Pediatr Neonat Individual Med. 2014;3(2):e030269. doi: 10.7363/030269. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. <http://creativecommons.org/licenses/by/3.0/>



In the primary phase of energy failure, reductions in cerebral blood flow, oxygen and or substrates, high energy phosphorylated compounds [such as the adenosine triphosphate (ATP) and phosphocreatine] have been observed, thus leading to tissue acidosis. The primary phase is recognized as an essential basis for all subsequent pathologic events. The primary energy failure is associated with acute intracellular derangements which included loss of membrane ionic homeostasis, release or blocked reuptake of excitatory amino acids and inhibition of protein synthesis (Johnston et al., 2001). In fetal sheep model, the resultant cerebral ischaemia has been shown to be associated with secondary cortical oedema and seizures, reduced final EEG power, loss of sleep state cycling, and significant loss of neurons and oligodendrocytes (Davidson et al., 2015; Wassink et al., 2015).

If the hypoxia-ischaemia event does not resolve (for example by maturation, substrate availability or body temperature), the injury can be aggravated, leading then to a secondary phase of energy failure (which has been estimated to be about 6 to 12 hours after the primary insult) which can last from hours to days (usually 12 to 48 hours) (Cowan et al., 2003; Jensen, 2006). The secondary phase

of energy failure is characterized by further declines in phosphocreatine and ATP without brain acidosis (Lorek et al., 1994). In this process, secondary neurotoxic mechanisms are activated; leading to the extracellular accumulation of excitatory amino acids or neurotransmitters (mainly glutamate) as a result of an increased release as well as impaired uptake.

As a consequent to this, over-activation of neuronal glutamate receptors, [mainly the N-methyl-D-aspartate (NMDA) receptor] occurs, which results in an excessive intracellular influx of calcium (accumulation). The accumulation of intracellular calcium causes activation of cell degrading enzymes (lipases, phospholipases, proteases and endonucleases) and the production of oxygen free radicals through activation of xanthine oxidase, increased prostaglandin synthesis, and activation of nitric oxide (NO) synthase.

In the secondary phase of brain injury, other adverse biological events include mitochondrial dysfunction (Kristian, 2004; Rousset et al., 2012; Taylor et al., 1999) and neuronal hyperexcitability which may or may not be associated with clinically evident seizures (depending on whether neurons are involved) (Jensen, 2009b). Accumulation of excitatory amino acids probably exacerbates the injury during the hyperexcitability of the neurons. The hyperexcitability in itself increases energy demands in the compromised brain, but in the absence of seizures, secondary neuronal loss can also occur. When the insult is removed, there is a phase of re-oxygenation, a phase also known as the reperfusion phase, which may be associated with the release of cytotoxins such as free radicals that can lead to cell destruction. The initial reperfusion phase can last from 10 to 30 minutes, resolving the primary intracellular oedema and restoring the cellular energy but neuronal activity remains depressed for some hours (Jensen, 2006).

In animal studies following resuscitation and restoration of cerebral perfusion, many surviving cells with depleted but not exhausted intracellular energy stores will begin to recover and rising energy stores may be observed. Unfortunately, this recovery is short-lived and cellular energy stores can be seen to deplete once again despite adequate cerebral perfusion resulting in secondary phase of energy failure (also known as delayed cell death) (O'Brien et al., 2006). This phase which consists of a fall in cerebral high-energy phosphate is also associated with a rise in intracellular pH.

Necrotic cell death is also another prominent process hypothesized in the immediate and acute phases of severe cerebral insults, with apoptosis

(programmed cell death) being the prominent mode of cell death during the secondary phase of injury (Northington et al., 2011). However, apoptosis is a biochemical, energy requiring process that can be inhibited at several points in the pathway by neuroprotective intervention.

Many effects induced by therapeutic hypothermia can help to reduce the number of cells undergoing apoptosis after hypoxic-ischaemia (Xu et al., 2002). The presence of a latent phase between primary and secondary energy failure also suggests that specific therapeutic interventions such as therapeutic hypothermia are possible to prevent brain injury through inhibition of the secondary neurotoxic mechanisms.

2.3.2 Stroke and seizures

To date, stroke is the second commonest identifiable cause of seizures in term neonates. Stroke occurs as a result of a sudden disturbance in the blood circulation (artery or venous) to the brain leading to a disruption in the function of the brain with pathological or radiological evidence of focal arterial or venous infarction (Kirton et al., 2011; Wu et al., 2005). Perinatal arterial ischaemic stroke (PAIS) occurs between 28 weeks gestation and 28 days of postnatal age, while neonatal stroke occurs within the first 28 days after birth for a term neonate or within term equivalent age of 44 weeks for a preterm neonate (Lynch et al., 2002). In the neonatal period, stroke is classified into 2 main types: ischaemic stroke and haemorrhagic stroke (Venkataraman et al., 2004).

Term neonates with arterial ischaemic stroke often present with seizures during the neonatal period even though they may have been considered healthy shortly after birth, with normal Apgar scores and cord pH values (Harteman et al., 2012; Mercuri et al., 1995). In the neonatal period, the most common clinical seizure observed in neonates with stroke is focal clonic in nature, involving the contralateral limb to the cerebral infarction.

Neonates with stroke are often non-encephalopathic. A study by Rafay *et al.* compared the EEG characteristics between neonates with PAIS and HIE (Rafay et al., 2009); they showed that there was no significant difference in the number of neonates who had electrographic seizures (PAIS vs HIE: 7 of 27 neonates vs 13 of 35 neonates; $p=0.350$). In their study, of neonates with stroke, normal background EEG was reported in 14 of 27 neonates, background asymmetry was noted in 27 neonates, unilateral rolandic periodic slow waves in 2 of 27 neonates,

lateralised EEG findings in 9 of 27 neonates and midline EEG findings in 3 of 27 neonates. The presence of electrographic seizures was noted in 7 of 27 neonates. Their results were limited because EEG findings were described exclusively from EEG reports which did not enumerate the degree of seizure burden and the number of seizure events in neonates with PAIS.

Pathophysiology of stroke

The pathogenesis of perinatal stroke is complex and multifactorial. According to Marret S (Marret et al., 2001), the pathophysiological factors associated with perinatal cerebral strokes may include the following: impaired or absent blood flow, non-genetic risk factors, genetic diseases and the multifactorial physiopathology of perinatal/neonatal strokes. As reported in other immature animal hypoxia-ischaemia models, the evolution of the neonatal stroke injury is quite prolonged (Johnston et al., 2001; Nakajima et al., 2000).

Acute focal ischaemia in the brain is associated with a dense necrotic core in which primary neuronal death occurs. This core is surrounded by an ischaemic 'penumbra', which has some residual blood supply (Memezawa et al., 1992). The evolution of injury in the penumbra is associated with waves of depolarization which deplete remaining cellular energy reserves (Nedergaard and Hansen, 1993). If ischaemia is permanent, damage progressively extends from the core to the penumbra over a few hours under experimental conditions (Folbergrova et al., 1992).

The infarction in the brain is frequently on the opposite side of the body where the clinical signs and symptoms may be manifested (depending on which part of the brain is affected). Isolated leg seizures had been noted in a case of infarction of the anterior part of the region supplied by the middle cerebral artery (MCA) (Billard et al., 1982) and isolated upper limb seizures with posterior truncal of the middle cerebral artery stroke had been observed by this group. These findings correspond to the penumbra as a partially functional area excited by waves of depolarization (Govaert et al., 2009b). However, stroke-induced seizures are not always contralateral to the site of infarction (Filipek et al., 1987). It has been suggested that due to more extensive brain injury, seizure could not be generated. Other types of cerebrovascular lesions other than cerebral infarcts may have contributed to the genesis of seizures (Aso et al., 1990).

There is evidence that the time window between ischaemia and the development of brain infarction in stroke patients may extend beyond 48 hours (Heiss et al., 1992). In 6 term neonates, Rutherford *et al.* showed that in the early phase of infarction (of up to 2 months), low signal areas with clearly defined margins developed at the site of infarction (Rutherford et al., 1997). In the late phase (from 2 months onwards) growth was seen in the brain at the margins of the infarction with the infarcted region showing a marked decrease in size (Rutherford et al., 1997). Although in some cases the rate of growth into the infarction appeared greater than general rate of growth of the brain, in other cases the rate of growth at the infarct margins was less than that of the brain as a whole. Growth of the undamaged tissue may provide an important mechanism for recovery of the developing neonatal brain.

Cerebral blood flow in neonatal stroke

Messer *et al.* observed that cerebral blood flow velocity values were completely absent or extremely reduced on the affected side of the middle cerebral artery in 2 patients with unilateral neonatal cerebral infarction during the first months of life (Messer et al., 1991). Perlman *et al.* also demonstrated the transient decreases in cerebral blood flow velocity on the affected side compared with the contralateral unaffected side of the middle cerebral artery in neonates with cerebral infarction (Perlman et al., 1994).

Using a transcranial Doppler, Nishimaki *et al.* showed that the systolic and diastolic blood flow velocities were increased but the resistance index values were markedly decreased on the affected side of the middle cerebral artery in the neonate who developed hemiplegia with cystic encephalomalacia (Nishimaki et al., 2001). The authors speculated that the asymmetry in resistance index values may result from the focal perfusion after focal ischaemic brain injury by cerebral infarction. They concluded that the asymmetry of the cerebral blood flow velocity and resistance index values in the neonatal period may be useful to evaluate the severity of brain injury and predict later neurodevelopmental outcome of unilateral neonatal cerebral infarction in neonates.

It has been shown that a large porencephalic cyst is the typical evolution of an early infarction in the territory of the middle cerebral artery, with or without the involvement of basal ganglia, a diffuse atrophy of the hemisphere and the sign of a secondary Wallerian degeneration of the cortico-spinal pathway at the brainstem level (Gunther et al., 2000). These findings (i.e. basal ganglia and internal capsula

involvement and Wallerian degeneration), have an adverse prognostic value to surrogate long-term neurodevelopmental outcome for possible evolution of hemiplegic cerebral palsy (Gunther et al., 2000)

The left middle cerebral artery in neonatal stroke

Neonatal strokes are often arterial in origin and ischaemic in nature (DeVeber et al., 2001). The distribution of cerebral infarction differs with gestational age. The lesions associated with stroke in the preterm neonates are often multifocal, involving the cortical and the lenticulostriate branches of the middle cerebral artery, rather than the trunk of the middle cerebral artery in term neonates (Barnette and Inder, 2009; de Vries et al., 1997). Like adult patients, unilateral infarctions of the left common carotid artery are more frequently (3 to 4 times) affected than the right in neonates (Estan and Hope, 1997; Govaert et al., 2000; Mercuri et al., 1999; Messer et al., 1991).

Approximately 75% of lesions occur on the left side of the brain (Levy et al., 1985; Perlman et al., 1994). The predominance of the left middle cerebral artery is poorly understood. The predominance of left sided lesions may be the result of vascular asymmetry (Trauner et al., 1993) or hemispheric differences in maturation and vulnerability (Uvebrant, 1988). Some authors have suggested a thromboembolic origin (Barmada et al., 1979; Ment et al., 1984; Nicolaidis and Appleton, 1996) where up to 54% of cases have been reported (Gunther et al., 2000). Others have suggested that emboli originating, either from the degenerating placental vessel before birth or in the just-activated pulmonary vascular bed after birth, have the ability to pass across the patent ductus arteriosus and access the carotid circulation (Coker et al., 1988).

The haemodynamic differences between the right and the left carotid arteries as a result of patent ductus arteriosus have been cited mostly as a possible cause for the predominance of stroke affecting the left side (Sreenan et al., 2000). The transient right to left intracardiac shunt, or from a more direct route through the left common carotid artery has been implicated (Sreenan et al., 2000). Contrary to this, focal ischaemic injury in neonates who have had extracorporeal membrane oxygenation occurred predominantly on the right side. This may be related to the right carotid artery which was often ligated during the procedure (Mendoza et al., 1991).

It is important to make an early distinction between the diagnoses in the neonatal period, as the approach to clinical management for stroke and hypoxic-ischaemic encephalopathy differs (table 2.4). There is currently sufficient evidence of benefit for the National Institute for Health and Clinical Excellence (NICE) to endorse the use of therapeutic hypothermia for hypoxic perinatal brain injury (National Institute for Health and Clinical Excellence (NICE), 2010). However, this has not as yet been recommended for stroke, albeit in the future, cooling may emerge as one of the potential treatment for perinatal stroke (Harbert et al., 2011; van der Worp et al., 2010).

Table 2.4 The comparative differences between neonates with stroke and hypoxic-ischaemic encephalopathy

| | Hypoxic-ischaemic encephalopathy | Stroke |
|---------------------------|--|---|
| |  |  |
| Cause of neonatal seizure | Most common | Second most common |
| Seizure onset | ≤ 24 hours | ≥ 12 hours |
| Standard of care | Cooled | Non-cooled |

Early brain imaging such as computed tomography (CT) and magnetic brain imaging (MRI) are of limited use to differentiate stroke and HIE in the immediate neonatal period because the affected neonates can be critically unstable for transport to the site where neuroimaging is located; requiring ventilation or cooling. Although, cranial ultrasound is readily accessible for most neonatologists, it is an insensitive tool to detect cerebral infarction at the early stages. Cranial ultrasound scans have been shown to have good diagnostic capabilities only when performed after day 4 after birth (Cowan et al., 2005), confirmation of diagnosis is only reliably achieved with MRI; however this facility is not readily available in many institutions.

The role of hypoxic-ischaemic encephalopathy in the pathogenesis of stroke in the perinatal period is controversial. Although stroke in the neonatal period has previously been attributed to perinatal asphyxia, ascribing a causal hypoxic-ischaemia event to the pathogenesis of stroke has been proven difficult (Cowan et al., 2003). Asphyxia with an element of hypoxia-ischaemia as a cause of perinatal arterial ischaemic stroke has been seen in less than 5% (6/134) of cases (Govaert et al., 2009a); however hypoxia-ischaemia is a rare cause for unilateral cerebral infarction.

2.4 Using anti-seizure medication

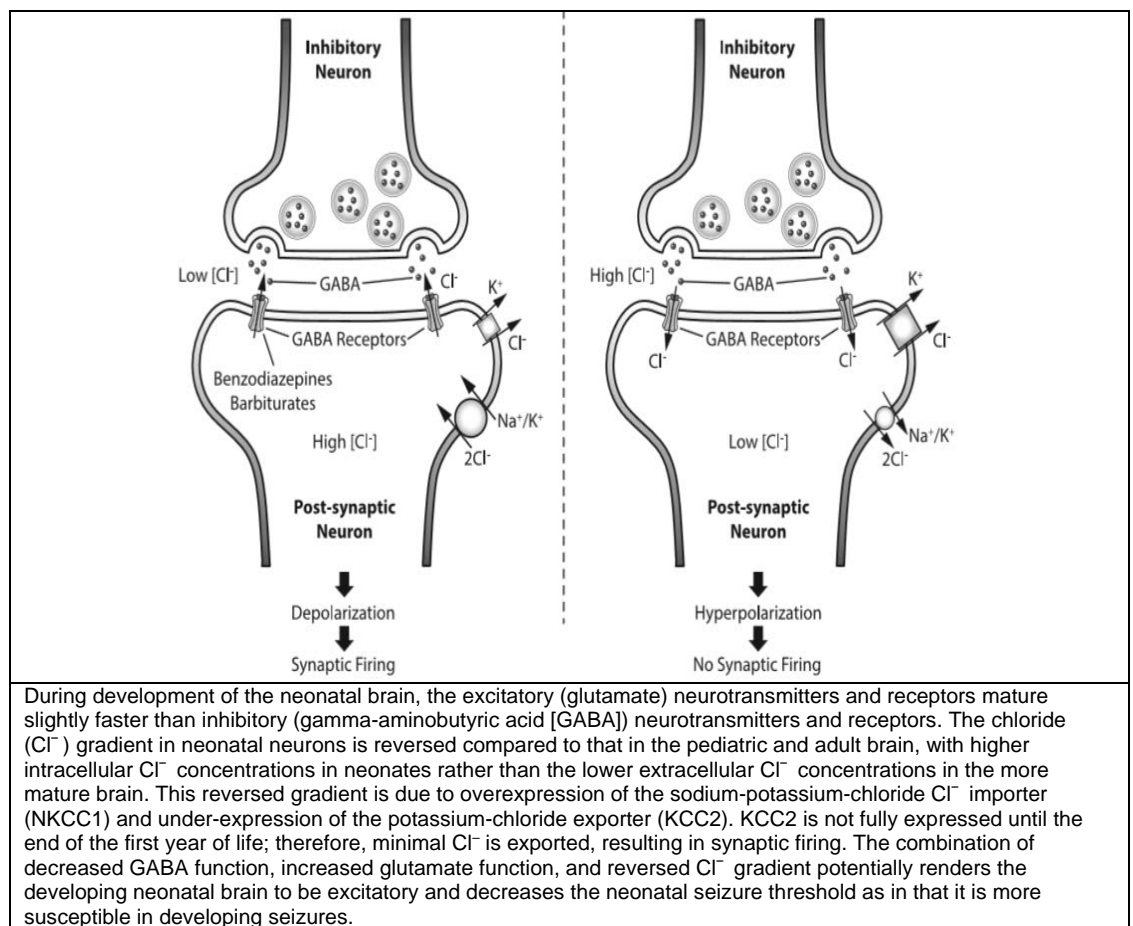
During both the pre and post-therapeutic hypothermia era, phenobarbitone has remained the most commonly used first-line anti-seizure medication for the treatment of neonatal seizures (Bartha et al., 2007; Hellstrom-Westas et al., 2015; Vento et al., 2010), despite being shown to be effective only in approximately 50% of cases (Boylan et al., 2002; Painter et al., 1999). Many investigators using animal models have shown that GABA is excitatory in the developing brain (Khazipov et al., 2004).

The developing neonatal brain may be resistant to GABA agonist such as phenobarbitone as a result of the higher concentration of intracellular chloride and because of the lower expression of the GABA receptors; both of which may account for the lesser sensitivity to benzodiazepine when compared to the adult brain (figure 2.5). This ineffectiveness has also been hypothesized to be related to the immaturity of neurotransmitters such as gamma-aminobutyric acid (GABA) in the developing neonatal brain (Jensen, 2009a).

Phenobarbitone, a barbiturate is believed to prolong the action of GABA (hence a GABA agonist) acting mainly on the GABA_A receptors in the adult brain model (Jones AW et al., 1950). To date, only 19 subunits constituting the GABA_A receptors have been discovered in the human adult brain (Sieghart et al., 2012); hence the complete structure of the GABA_A receptor has not yet been fully deciphered (Loscher and Rogawski, 2012), let alone in the developing neonatal brain. Some GABA_A receptors may be present in the neonatal brain; experiments have shown that phenobarbitone was only selective for the neocortex (Olsen RW, 2002), particularly in certain parts of the thalamus at very high and potentially toxic doses (Mathers et al., 2007). Interestingly in the adult brain, it is speculated that phenobarbitone may activate the GABA_A receptor through different mechanisms by inducing different conformational changes in the GABA_A receptor structure (Eaton et al., 2012; Mercado and Czajkowski, 2008; Muroi et al., 2009); this uncertainty may also occur in the developing neonatal brain.

Figure 2.5 The difference between neonatal and adult based on neuronal chloride gradient and the mechanism of action by GABA agonist (Mruk et al., 2015)
Used with permission of the copyright holder, © 2015 Pediatric Pharmacy Advocacy Group. First published in Mruk AL, Garlitz KL, Leung NR. Levetiracetam in neonatal seizures: a review. *J Pediatr Pharmacol Ther.* 2015;20:76-89. Available at: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4418685/> (Accessed 12 January 2016)

| Neonatal | Mature |
|----------|--------|
|----------|--------|



If GABA agonists facilitate further seizures in the immature developing brain, then phenobarbitone should increase seizures around the time when is administered during ongoing seizures. Yet, this is never seen in clinical practice. GABA antagonists however, have not been shown to reduce seizures (Moshe, 1987). Most of what we understand about the paradoxical effect of GABA in the developing brain is derived from animal studies and these have merit, but the clinical situation in the human neonate might be very different and more complex than currently hypothesized in the literature (Ben-Ari et al., 2007; Ben-Ari, 2012).

Additional anti-seizure effects of phenobarbitone have been shown to be associated or interlinked to its ability to inhibit some voltage-activated ion channels particularly voltage-activated calcium channels (Schober et al., 2010) and to block non-N-methyl-D-aspartate receptors (Nardou et al., 2011a). Therefore the anti-seizure effect of phenobarbitone is not likely to be explained solely by its effect on the GABA_A receptors, and its ability to block other ion channels cannot be dismissed in the developing neonatal brain. Furthermore, to know how phenobarbitone precisely works as an anti-seizure medication, future research should delve into unearthing the precise location for its binding sites on the GABA_A receptor, its action on other ion channels or other possible mechanisms in the developing neonatal brain.

Other unidentifiable subunits or alternative inhibitory systems may be implicated, creating a pathway for phenobarbitone to be effective in reducing seizure burden in the early neonatal period and in the Phenobarbitone study (discussed in greater detail in Chapter 8). At later stages of seizure progression during recurrent seizures, Nardou *et al.* hypothesized that the change (increase) in the concentration of intracellular levels of chloride may enhance the excitatory component of GABA, causing GABA agonists such as phenobarbitone to be ineffective in reducing seizure burden (Nardou et al., 2011b).

Another possible reason may be due to the pharmacoresistance of phenobarbitone acting on the GABA_A receptors when phenobarbitone is given at a later stage of ongoing seizures (Jones et al., 2002). Based on an adult rodent model of status epilepticus treated with diazepam versus phenobarbitone, Jones *et al.* (Jones et al., 2002) found that the abolition of seizure was expedited when phenobarbitone was instigated at less than 10 minutes compared to more than 10 minutes from onset of either clinical or EEG seizures. To explain the theory on pharmacoresistance of phenobarbitone towards GABA, 4 possible hypotheses were offered: a change in the GABA subunit (loss of $\gamma 2$ subunit replaced by δ subunit of GABA_A receptor); activation of a non-functional 'spare' GABA_A receptor; uncoupling of receptors (relating to the massive release of GABA during ongoing seizures) and the post-translational modification of GABA_A receptor (Jones et al., 2002). Whether these mechanisms apply to the developing neonatal model are yet to be investigated.

Alternatively, for phenobarbitone to exert its anti-seizure ability, a certain degree of inhibition in the brain may be required. Although there have been many theories hypothesizing the excitatory action of GABA present in the neonatal brain, some inhibitory action of GABA in the developing neonatal brain has been demonstrated as early as during the first postnatal week (Isaev et al., 2007; Tyzio et al., 2006). In rodents, the release of GABA originating from the hippocampus has been shown to cause inhibition (Dzhala et al., 2012; Wong et al., 2005). These studies suggest that phenobarbitone indeed has the ability, to some degree, to reduce seizures in the immature brain. Therefore, further studies are required to investigate the inhibitory action of GABA in the human neonatal brain which will shed further light on why phenobarbitone is able to reduce seizures if administered soon after seizure onset.

During the initial stage of treatment, the response of seizures to first-line anti-seizure medication is unpredictable, prompting the use of second-line anti-seizure medication in most cases if there were persistence of clinical seizures (Glass et al., 2012; Malone et al., 2009; van Rooij et al., 2013b; Wickstrom et al., 2013). However, there is variation as to the choice of the second-line anti-seizure medication (Boylan et al., 2004; Hellstrom-Westas et al., 2015). There is still no consensus among neonatologists today as to what incremental dose of phenobarbitone and which type of second or third-line anti-seizure medication should be appropriate when first-line anti-seizure medication such as phenobarbitone failed to control seizures in neonates (Hellstrom-Westas et al., 2015; van Rooij et al., 2013a).

A recent international survey revealed that more than 70% of neonatologists, neurologists and specialists in neonatal neurocritical care still use phenobarbitone as the first-line anti-seizure medication (Bartha et al., 2007; Glass et al., 2012), more than 40% used phenytoin as second-line and more than 13% used lorazepam as third-line anti-seizure medication to treat neonatal seizures (Glass et al., 2012). This practice is based on tradition and individual protocol rather than on evidence-based medicine. There is no consensus on the optimal time to discontinue anti-seizure medication (Bartha et al., 2007; Glass et al., 2012; Hellstrom-Westas et al., 2015; Wickstrom et al., 2013). Treatment of neonatal seizures needs to reflect effectiveness and perhaps we need to change our current strategy of treating neonatal seizures in our neonatal units.

Bumetanide is a loop diuretic which has been proposed as an adjunct to GABAergic drugs like phenobarbitone to help overcome the depolarizing action of immature neurons to GABA agonists (Cleary et al., 2013). Further experimental work has shown that when bumetanide (a NKCC1 antagonist) is used, it reduces intracellular chloride leading to the attenuation from the normally excitatory response of immature cells with high NKCC1 expression to an inhibitory response (Glass, 2014; Khanna et al., 2013), and suppresses seizures (Dzhala et al., 2010). It has also been shown to enhance the action of phenobarbitone in neonatal rats (Cleary et al., 2013).

Bumetanide blocks the excitatory nature of GABA by reversing the chloride gradient, phenobarbitone then enhances the GABA receptors to maintain that chloride current in the channels. However, Puskarjov *et al.* suggested that since bumetanide remains suboptimal due to its lack of target specificity, studies focusing on developing more specific NKCC1 inhibitors with its increased in

central nervous system penetration, direct and indirect strategies to enhance KCC2-mediated neuronal chloride extrusion, may pave the way to better therapeutic modulation of the GABAergic system for the treatment of neonatal seizures (Puskarjov et al., 2014). Further work should investigate whether bumetanide has any merit when used as an adjunct with phenobarbitone in the neonatal intensive care unit (Pressler et al., 2015; Pressler and Mangum, 2013).

Levetiracetam is potentially a useful anti-seizure medication in neonates despite its limited data on its efficacy (Glass et al., 2012; Silverstein and Ferriero, 2008). Levetiracetam has been shown to be an effective anti-seizure medication by reducing more than 50% of the seizure burden within 24 hours in 8 of 23 neonates with no adverse effects (Abend et al., 2011). The mechanism of action of levetiracetam continues to be evaluated and has not been fully elucidated. Levetiracetam is a pyrrolidine derivative antiepileptic that binds to the synaptic vesicle protein synaptic vesicle glycoprotein 2a (SV2a), which is expressed throughout the brain. When levetiracetam is binded to SV2a, neurotransmitter release and vesicle transport are impeded within the neuron (Talos et al., 2013; Yang and Rothman, 2009). Because the SV2a is found in all areas of the brain, it can treat partial seizures that arise in various regions of the brain, as seen in neonatal seizures. Unlike phenobarbitone and phenytoin, levetiracetam was shown to be devoid of proapoptotic actions in animal models (Forcelli et al., 2012; Kim et al., 2007; Manthey et al., 2005).

Midazolam is one of the most widely used sedatives in the neonatal intensive care unit and is used when seizures are refractory to phenobarbitone use (Castro, Jr. et al., 2005; Hu et al., 2003; Sheth et al., 1996; Sirsi et al., 2008). The sedative and anti-seizure properties of midazolam are related to GABA accumulation and occupation of benzodiazepine receptors (Pacifici, 2014). In a non-randomized study, all seizures were rapidly controlled with midazolam in 13 non-responders to phenobarbital/phenytoin (Castro, Jr. et al., 2005). Sirsi *et al.* reported status epilepticus due to different aetiologies in three neonates who did not respond to phenobarbital and phenytoin but responded to a midazolam infusion (Sirsi et al., 2008).

Lidocaine is also widely used for refractory neonatal seizures in most neonatal units (Lundqvist et al., 2013; Malingre et al., 2006; van den Broek et al., 2013). Some authors have indicated that lidocaine is an effective drug for refractory seizures as second- or third-line treatment with response rate varying from 70% to 92% (Shany et al., 2007; Yamamoto et al., 2007). In a study by Boylan *et al.*, three

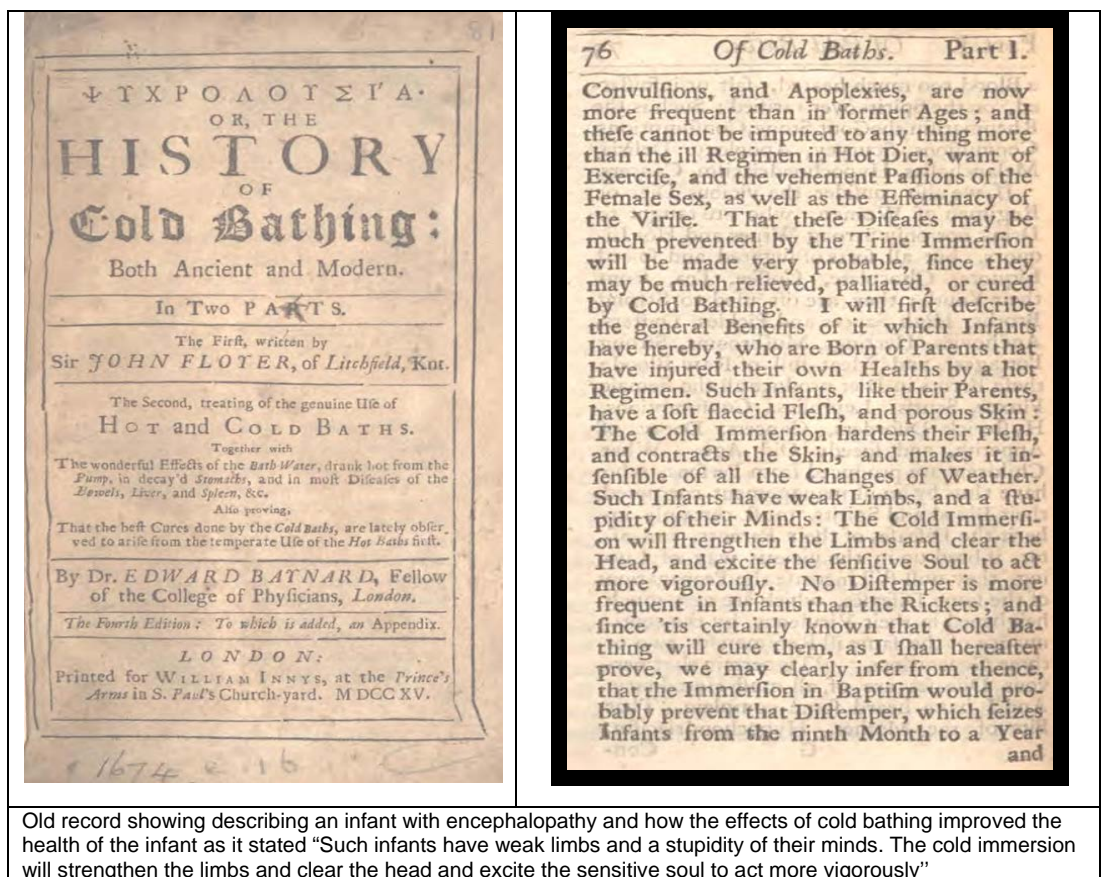
of five infants responded to lidocaine as a second-line drug after phenobarbital (Boylan et al., 2004).

Topiramate has been shown to have multiple mechanisms of anticonvulsive action in animal models of seizures and brain injury (Cha et al., 2002; Liu et al., 2004), and has been shown to be an effective neuroprotective agent with its safety and efficacy established as an anti-seizure medication in neonates (Glass et al., 2011c). A recently developed intravenous preparation of topiramate has been shown to be well tolerated in adult volunteers; it has been shown to have equivalent bioavailability to the oral formulation and this anti-seizure medication is promising for use in neonates (Clark et al., 2013).

2.5 Using therapeutic hypothermia to treat neonatal seizures

Cold, as a simple and easily accessible element, is among man's earliest remedies (Wang et al., 2006). The use of cold as a therapeutic agent has had a long and colourful history in both medicine and surgery (Wang et al., 2006). The concept of hypothermia as a treatment of brain injury is not new (Floyer J, 1674) (figure 2.6). The use of hypothermia in the treatment of asphyxia was suggested 65 years ago by JA Miller Jr. whose treatment was based on a well-known fact that rates of chemical reactions including those involved in living processes depend upon temperature (Miller JA. et al., 1964). Since 1884, this is known as the Van't Hoff' rule (van't Hoff JH, 1884) which states that the rates of chemical reactions increase two times or more for each 10°C rise in temperature.

| Figure 2.6 Ancient records on cooling in infants (Floyer J, 1674) | |
|---|--|
| a) Record of cooling in the 17 th century | b) Description of a neonate with HIE in the 17 th century |



In the 1960s, Miller and Westin (Miller JA. et al., 1964) studied the physiologic basis for the neuroprotective role of hypothermia in the treatment of "asphyxia neonatorum," firstly in newborn animals and then in human newborns. When conventional resuscitation techniques failed, they demonstrated an improved survival without cerebral palsy or mental retardation when neonates that were apnoeic after delivery were cooled rapidly to core temperatures of 23 to 32°C. Bernard *et al.* provided the preliminary observations in which treatment with moderate hypothermia appears to improve outcomes in adult patients with coma after resuscitation from out-of-hospital cardiac arrest (Bernard et al., 2002). Cerebral reperfusion injury occurs when cerebral blood flow is restored after cardiac arrest and resuscitation.

Perinatal asphyxia is one of the most damaging of neurologic processes and remains an important cause of long-term neurodisability and death (Edwards et al., 2010). The timeframe in which hypothermia used as a treatment option has often been closely discussed together with the mechanism of brain injury incurred by hypoxic-ischaemic encephalopathy and this is discussed below.

Effects of cooling on seizures

At the molecular level, cooling can directly or indirectly affect the key roles of ion channels, particularly voltage-gated sodium channels involved in seizure generation (Motamedi et al., 2013). Increased intracellular levels of glutamate (which is an excitatory neurotransmitter released from presynaptic terminals) activate ion-channel complexes that cause calcium to shift from the extracellular to the intracellular fluid, leading to the accumulation of oxygen free radicals and the activation of degradative enzymes which are damaging to the brain.

Neuroprotection occurs when hypothermia reduces the glutamate level and the subsequent production of oxygen free radicals (Nakashima and Todd, 1996).

Further at the molecular level, hypothermia reduces neuronal activity, decreases energy requirements for intrinsic cellular support and membrane homeostasis (Bennet et al., 2001; Nakashima and Todd, 1996; Tooley et al., 2003), and reduces the cerebral energy metabolism during the primary injury phase, thus delays the progression of primary damage and alleviates post-reperfusion injury. Some studies have shown that cooling markedly delayed apoptosis even when it does not completely suppress it (Azzopardi et al., 2009; Gunn et al., 2005). Wassink *et al.* suggested that there may be secondary processes pioneering a cascade of deleterious events involved in the "execution" phase of cell death (Wassink et al., 2014); these events may explain some of the morbidity and mortality observed in neonates with hypoxic-ischaemic encephalopathy who had received therapeutic hypothermia.

In animal studies, several experiments have demonstrated the effects of hypothermia on seizures (Bennet et al., 2001; Busto et al., 1989; Globus et al., 1995; Nakashima and Todd, 1996; Tooley et al., 2003). In fetal sheep, hypothermia was associated with a marked reduction in the amplitude of seizures and other epileptiform activities in the first 6 hours after a complete umbilical cord occlusion (Bennet et al., 2001). In a piglet model of asphyxia, the duration of individual electrographic seizures were reduced in the cooled group when compared to the non-cooled group (Tooley et al., 2003). Hypothermia to 30 or 33°C has been shown to completely inhibit the release of glutamate in a rat model of cerebral ischaemia (Busto et al., 1989). Other effects of hypothermia such as reduced cytotoxic oedema by reducing amino acid release (Nakashima and Todd, 1996) and inhibition of free oxygen radicals (Globus et al., 1995), may have an impact on the reduction in seizure burden. Whether the amplitude, morphology

and distribution of electrographic seizures in cooled neonates differ from that in non-cooled neonates will require further investigation.

In a rodent study, rapidly cooling the cortex to 20 to 25°C as quickly as possible after seizure onset and maintaining cooling for 0.5 to 2 minutes, showed that the average seizure duration was dramatically reduced by 90% (Hill et al., 2000). Cooling reduced seizure duration from 68.7 ± 18.7 seconds to 42.8 ± 13.9 seconds ($p < 0.05$) and seizure frequency (total number of subsequent seizures during a 70 minute observation period) from 20.6 ± 10.7 to 6.4 ± 6.2 ($p < 0.05$). After two periods of cooling, the frequency and duration of subsequent seizures were significantly reduced. This effect observed in in-vitro experiments suggests that cooling might have other anti-seizure effects (Hill et al., 2000).

Rewarming seizures or seizures following discontinuation of therapeutic hypothermia

With the advent of therapeutic hypothermia, neonatologists now have to deal with rewarming seizures, but there are only a few reports on this so far (Battin et al., 2004; Kendall et al., 2012; Shah et al., 2014). Rewarming seizures can be common (Battin et al., 2004; Shah et al., 2014) and can continue unabated even after the rewarming period (Kendall et al., 2012). There is no direct explanation as to why seizures occurred during rewarming. However, the main hypothesis which has been postulated is that rewarming seizures may be due to the re-accumulation of chemicals which are involved in the seizure generation pathway leading to the re-ignition of the seizure pathway; which had been either in a state of decrease or stagnant production or under-expressed during cooling. In a rabbit model which were cooled to a core temperature of 33°C, a decrease in nitric oxide production and hippocampal cell loss were noted during kainate-induced seizures (Takei et al., 2005). During rewarming, there was an increased in nitric oxide production in the hippocampus during seizures (Takei et al., 2005).

The incidence of rewarming seizures remains speculative. Although there has been valid explanation in theory of its pathophysiology suggesting that it can be a common occurrence, it remains a rarity and anecdotal in clinical practice (Battin et al., 2004; Gerrits et al., 2005; Shah et al., 2014). Perhaps one of the reasons to this is because most neonatal seizures are subclinical, and that without EEG monitoring, rewarming seizures may have been missed and remain undetected. Although some studies have speculated that rewarming seizures are benign (Kendall et al., 2012; Shah et al., 2014), further studies are required to establish their significance.

Seizure recognition by clinical observation and aEEG during cooling

Previously published neonatal hypothermia trials could not accurately measure seizure burden as their protocols did not include early, prolonged and continuous multichannel EEG monitoring. These studies used clinical (Kwon et al., 2011) and/or aEEG monitoring (Edwards et al., 2010; Simbruner et al., 2010) for seizure recognition. The recently published Neonatal Research Network Whole Body Hypothermia Trial relied on clinical recognition of seizures only (Kwon et al., 2011) and when the authors adjusted for hypothermia and severity of encephalopathy, hypothermia did not appear to have any impact on the frequency of clinical seizures and outcome. However, clinical estimation of seizure burden is notoriously unreliable with the majority of neonatal seizures being subclinical (Malone et al., 2009; Murray et al., 2008). Subclinical seizures have been reported to be prevalent even in neonates treated with therapeutic hypothermia (Yap et al., 2009).

When available in some participating neonatal institutions in the Total Body Hypothermia for Neonatal Encephalopathy (TOBY) trial (Azzopardi et al., 2009), the aEEG (not restricted to any number of channels) had been used for recruitment and as a monitoring tool during therapeutic hypothermia. At recruitment, clinical seizures and seizures detected by aEEG (did not specify how many channels were used) were present in 67% (74 or 110) of neonates and 29% (33 of 115 neonates) of neonates respectively. The TOBY trial considered seizures as a complication during therapeutic hypothermia, with a decreasing incidence from day 1 to 4 (90% to 23%). Both clinical recognition of seizures and the aEEG are known to underestimate the actual true seizure burden (Boylan et al., 2013). The aEEG cannot detect short seizures, seizures which do not generalize and low voltage seizures (Boylan et al., 2013; Rennie et al., 2004). Furthermore, there is also a degree of inter-observer variability in aEEG interpretation (Boylan et al., 2013; Rennie et al., 2004; Shellhaas et al., 2007).

At present, the TOBY registry lead by Azzopardi *et al.*, has not made brain monitoring as a prerequisite for cooling (Azzopardi D et al., 2007). They had recommended that if possible, some form of cerebral function monitoring be performed on neonates receiving therapeutic hypothermia either before the induction of cooling or as soon as possible during cooling. Prolonged monitoring should be extended long after cooling has been discontinued as rewarming seizures may go undetected without EEG monitoring, and that the multichannel EEG monitoring is crucial to detect electroclinical dissociation of seizures. A longer

EEG recording time will increase the possibility of capturing more seizures and using multichannel EEG will not miss seizures arising from other cerebral regions of the brain, which will inherently be missed when monitoring with the aEEG (which uses the limited electrode placement).

2.6 Using anti-seizure medication during therapeutic hypothermia to treat neonatal seizures

Although the use of phenobarbitone as a monotherapy has been shown to be ineffective for the treatment of seizures in many neonates, phenobarbitone has been rendered more effective when co-administered with other forms of treatment such as with therapeutic hypothermia (Barks et al., 2010). In a rodent study, phenobarbitone was shown to augment the therapeutic effect of cooling (Barks et al., 2010). As an anti-seizure medication, phenobarbitone has the potential to reduce endogenous heat production and thus exaggerate the fall in temperature during active cooling. In the setting of therapeutic hypothermia, phenobarbitone has been shown to contribute to neuroprotection by decreasing the antioxidant effects, decrease cerebral oedema and cerebral metabolic demand (Barks et al., 2010), which may in turn exert its anti-seizure effects.

It is known that the half-life of phenobarbitone is significantly increased when neonates are treated with hypothermia (Filippi et al., 2011), and with reduced hepatic metabolism during hypothermia, plasma drug levels will accumulate (Roka et al., 2008). The bioavailability of phenobarbitone in neonates is usually longer ranging from 45 to 500 hours (Takemoto CK et al., 2012); it can be variable depending on circumstances (e.g.: renal and hepatic enzyme excretion and metabolism, drug distribution and clearance during therapeutic hypothermia) (Faught, 2001; Filippi et al., 2011; Shellhaas et al., 2013; van den Broek et al., 2012) and is different from adults (Marsot et al., 2013). Van den Broek *et al.* assessed the pharmacokinetics of phenobarbitone in a cohort of 31 neonates (≥ 36 weeks gestation) with HIE who were cooled (van den Broek et al., 2012). The authors advocate the use of up to 40 mg/kg of phenobarbitone in total before proceeding to a second-line anti-seizure medication as plasma levels remained below therapeutic range during therapeutic hypothermia. Based on a study undertaken before the era of therapeutic hypothermia, phenobarbitone doses higher than 40 mg/kg have been shown to increase neuronal apoptosis (Gilman et al., 1989).

In a mouse model, therapeutic hypothermia and histone deacetylase inhibitors, such as valproic acid, independently have been shown to have neuroprotective properties in models of cerebral ischaemic and traumatic brain injury (Jin et al., 2014). Hypothermia has been shown to increase the blood concentration of anti-seizure and anaesthetic drugs (Filippi et al., 2011; Tortorici et al., 2007). The particular dosing of anti-seizure medication during cooling may have to be further investigated to assess whether the dosing is optimal. Furthermore, pharmacokinetic data in neonates differs significantly from older children and adult (Allegaert et al., 2008); normative neonatal data is not available, as a placebo group of non-cooled neonates is not ethically possible to obtain nowadays.

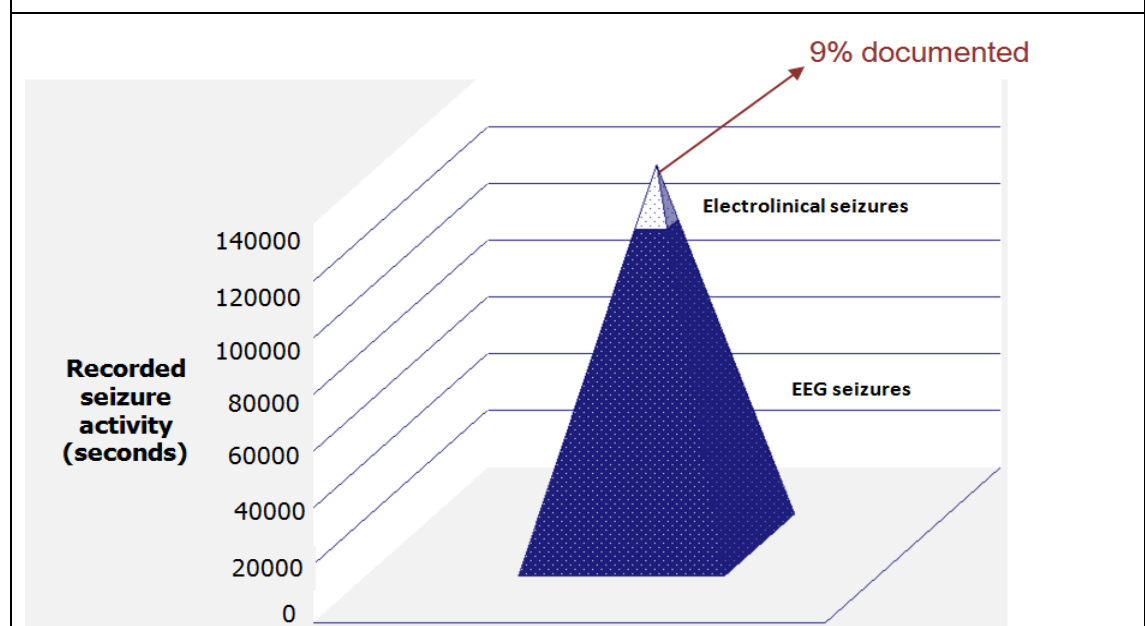
Sedative and anaesthetic drugs have also been shown to facilitate the therapeutic effects of hypothermia (Tooley et al., 2003). Based on the timing of hypoxia-ischaemia and in the setting of hypothermia, there are many potential and possible anti-seizure medications which have been hypothesized to work. Lidocaine has been shown to be neuroprotective (van den Broek et al., 2011) and xenon, a potent anaesthetic agent has been shown to reduce seizures (Azzopardi et al., 2013; Lobo et al., 2013) when combined with cooling (Thoresen et al., 2009).

Clinical Manifestation and Detection of Neonatal Seizures

Introduction

In neonatal units worldwide, clinical detection of neonatal seizure is an ongoing problem and remains a challenge for nursing and medical personnel. For parents and families, witnessing a seizure is a devastating experience. Nonetheless, some neonates may display clinical behaviours which are suggestive of seizures but do not have an electrographic correlate. Early and accurate recognition of seizures in neonates through cotside training of nursing and medical personnel is an important step for the clinical management of neonatal seizures (Glass et al., 2010). However in the neonate, seizure recognition and physical examination have proven inadequate and unreliable among nursing and medical personnel (Malone et al., 2009; Murray et al., 2008) (figure 3.1).

Figure 3.1 Clinical neonatal seizures detected clinically are only the tip of the iceberg (adapted from (Murray et al., 2008))



Seizure detection with continuous electroencephalogram monitoring is more accurate than clinical observation, but it required interpretation from specialized experts which may not be available in most neonatal intensive care units (NICUs). As a result to date, a more popular method to assess and monitor cerebral function in sick neonates is to use the amplitude-integrated EEG (aEEG), which is a simplified method of EEG recording, which is based on easy-to-interpret pattern recognition of compressed EEG output trends, generated from one or two channels. However, it can be problematic with errors for clinical interpretation as

short duration and low amplitude seizures can be missed, artefacts which can look like seizures may be misinterpreted as actual seizures; thus treatment of seizures may be misguided and suboptimal. Some authors have found that seizure detection with the use of the aEEG has been proven to be disappointing, albeit its sensitivity has been quoted to be close to 80% in some studies where experienced raters were used (Shah et al., 2008; Shellhaas et al., 2007; Toet et al., 1999). To optimize brain monitoring in neonates who are at high risk of developing seizures, multichannel EEG monitoring is required. A promising alternative centers on the development of a neonatal automated seizure detection algorithm (NASDA) which ultimately may provide the solution to these perplexities (Boylan et al., 2015; Boylan and Rennie, 2006).

3.1 Manifestation of neonatal seizures

Physically, neonatal seizures have been defined as abnormal, stereotyped, paroxysmal alterations in neurological function(*i.e.* motor, autonomic or behavioural) (Volpe JJ, 2008), in the first 28 days after birth in term neonates, or before 44 weeks of gestational age in preterm neonates (Thibeault-Eybalin et al., 2009). However, the accurate definition of neonatal seizures can no longer rely on the classification of clinical manifestations; multichannel EEG is required to detect all neonatal seizures and remains the gold standard for the detection of neonatal seizures. This implies that the recognition and quantification of seizures in neonates rests solely on the gold standard of seizure detection. Furthermore, the Neurology Group on Neonatal Seizures has recommended the use of continuous video-EEG monitoring as a requirement to establish the presence and the number of seizures (Clancy, 2006b).

With or without clinical manifestations, electrographically (multichannel EEG is required) documented seizures represent the most accurate way to detect and quantify neonatal seizures. Four main definitions are required for neonatal seizures:

1. Clinical seizures: These are clinically observed seizures, which may include paroxysmal changes in neonatal activity such as behavioural or autonomic function, which may be correlated with EEG changes (Volpe JJ, 2008).
2. Electroclinical seizures: These are clinical seizures which are accompanied by EEG seizure discharges. In other words, they are seizures with both clinical features and electrographic correlates (Boylan et al., 1999).

3. Electrographic seizures or EEG seizures (multichannel EEG is required): These are seizures seen on the EEG as seizure discharges (Tekgul et al., 2005), possessing characteristic features (stereotyped, evolving, repetitive waveforms) and duration. Further discussion is found in Chapter 4 entitled “The Neonatal EEG and Electrographic Seizures”.
4. Electroclinical dissociation (ECD) of seizures: These are electrographic seizures that are not consistently accompanied by clinical manifestations (Boylan et al., 1999; Volpe JJ, 2008; Weiner et al., 1991). Usually, they occur in neonates with diffuse encephalopathies, those who have received anti-seizure medication, particularly barbiturates and in sedated neonates (Tharp, 2002).

3.1.1 Electroclinical seizures: clinical seizures with EEG correlates

3.1.1.1 Clonic seizures

Physically, these seizures are rhythmic limbs movements consisting of approximately 1 to 3 jerks per second at the onset, with the rate progressively declining over time (Volpe JJ, 2008). They can be classified as focal, multifocal or generalized. Focal unilateral clonic seizures (left or the right side) involve the face, neck, trunk, upper or lower limbs at any one time. Multifocal clonic seizures involve several parts of the face, body and limbs at the same time and often in a migrating fashion. Generalized clonic seizures involve bilaterally, symmetrically and synchronously movements of the body and limbs. Clonic seizures have been observed to be the most common type of clinical seizure, and they have been consistently associated with electrographic seizures (Mizrahi EM and Kellaway P, 1998).

3.1.1.2 Tonic seizures

These seizures can be categorized as focal or generalized. Focal tonic seizures have been described as the sustained posturing of a limb or asymmetrical posturing of the trunk and neck. Tonic seizures have also noted to be associated with electrographic seizures (Mizrahi and Kellaway, 1987; Shellhaas and Clancy, 2007). Generalized tonic seizures are characterized most commonly by tonic extension of both upper and lower limbs, (often resembling “decerebrate” posturing), followed by tonic flexion involving the upper limbs together with the extension of the lower limbs (often resembling “decorticate” posturing). Approximately 85% of generalized tonic movements are not accompanied by electrographic seizures (Mizrahi and Kellaway, 1987).

3.1.1.3 Myoclonic seizures

Myoclonic seizures are distinguished from clonic seizures because of their faster speed (approximately more than 1 to 3 jerks per second) (Volpe JJ, 2008) and because they have a predilection for the flexor muscle groups. Myoclonic seizures can be classified into 3 categories: focal, multifocal and generalized. Focal myoclonic seizures characteristically involve flexor muscles of an upper limb. Multifocal myoclonic seizures are characterized by asynchronous twitching of several parts of the body.

Generalized myoclonic seizures are characterized by bilateral jerks or flexion of the upper and lower limbs. Generalized myoclonic seizures are more likely to be associated with electrographic seizures than focal or multifocal myoclonic seizures. Conditions which are commonly associated with myoclonic seizures are benign sleep myoclonus (Paro-Panjan and Neubauer, 2008), familial neonatal seizures (Saadeldin et al., 2013) and conditions arising from inborn errors of metabolism (Vesela et al., 2009; Yu and Pearl, 2013).

3.1.1.4 Subtle seizures

The inconspicuous nature of this type of seizure often perplexes observers who rely merely on visual acumen for seizure identification (Malone et al., 2009; Murray et al., 2008). They include paroxysmal alterations in the behaviour of the neonate which can have a motor or an autonomic component. However, the motor component does not have a clear clonic, tonic or myoclonic feature. They are more commonly detected in preterm than in term neonates (Whitelaw, 2012). In a group of neonates between gestational ages of 26 to 32 weeks, subtle seizures have been described as sustained eye opening, ocular movements, chewing, pedaling motions and a variety of autonomic phenomena such as changes during apnoeic events (decreased in oxygen saturation levels, heart rates and respiratory rates) (Castro, Jr. et al., 2012; Sirsi et al., 2007).

3.1.1.5 Apnoeic seizures and EEG suppression

Apnoeic seizures in term neonates have been reported, and were mainly associated with subtle movements such as eye deviation, opening or staring and mouth movements (Miyagawa et al., 2007; Ramenghi et al., 2009; Sirsi et al., 2007). Based on a case report of 2 neonates with occipital infarction, it was postulated that the connections between the posterior limbic cortex and the temporal lobe with the midbrain respiratory centers may explain the presentation of apnoeic seizures (Castro, Jr. et al., 2012). Apnoeic seizures are commonly associated with temporal lobe haemorrhage in term neonates (Sirsi et al., 2007;

Tramonte and Goodkin, 2004), but they are not usually associated with changes in heart rate (Fenichel et al., 1980). However, apnoeic seizures accompanied by electrographic seizures (also known as convulsive apnoea) have been reported to be associated with bradycardia; convulsive apnoea more than 60 seconds may be complicated by bradycardia, which may be secondary to cerebral hypoxia (Fenichel et al., 1980). During apnoeic seizures, the temporal area has been prominently found to initiate ictal discharges; suggesting that the temporal lobe is involved in the limbic origin of apneic seizures (Watanabe et al., 1982).

Although abnormal movements mimicking seizures are sometimes seen in neonates who have apnoea, electrographic seizures during EEG monitoring are not observed. In fact, EEG suppression is more likely to be present during these apnoeic episodes (Low et al., 2012b). In fetal lambs, Gunn *et al.* has shown that the EEG becomes isoelectric during an ischaemia event (Gunn et al., 1992). Recovery of EEG activity depended on the duration of the ischaemic event; shorter durations of ischaemia tended to lead to full recovery of EEG activity. If the ischaemia lasted 30 minutes or longer, a stereotypic sequence of depressed EEG activity followed by low frequency epileptiform activity was always seen.

In the newborn piglet model, hypoxic-ischaemic events induced by reducing fractional inspired oxygen to around 6% has been shown to generate a rapid suppression of EEG activity. Brain injury was only seen when the EEG amplitude remained suppressed for 23 minutes or more (Thoresen et al., 1996). In another study where one week old piglets were subjected to graded hypoxia, the EEG amplitude did not decline until oxygen saturation fell below 25% (Gavilanes et al., 2004). This is similar to the effects described in animal studies when hypoxia has been used to induce severe EEG suppression (Sanocka et al., 1988). In piglets, EEG amplitude has been shown to decrease markedly after approximately 30 seconds of apnoea induced by stimulation of the superior laryngeal nerves (Sanocka et al., 1988).

3.1.2 Electroclinical dissociation (ECD) of seizures

Electroclinical dissociation is an event when electrographic seizures are not consistently accompanied by clinical manifestation; the majority of neonatal seizures has been described as mainly of this nature (Boylan et al., 1999; Zangaladze et al., 2008). The persistence of EEG seizures has also been termed as “decoupling” or “uncoupling” (Bye and Flanagan, 1995a; Connell et al., 1989; Scher et al., 2003); it has also been defined as the persistence of electrographic

seizures despite the suppression of $\geq 50\%$ clinical seizures, after either one or more anti-seizure medication were used (Scher et al., 2003).

Electroclinical dissociation of seizures at the molecular level

Electroclinical dissociation (ECD) of seizures in term neonates may be due to regional interconnectivity, including interhemispheric as well as corticospinal, which are not fully mature due to incomplete myelination of white matter tracts, leading to only modest or no behavioral manifestations of these seizures. Neonates can show no signs or very subtle tonic or clonic movements, often limited to only one limb, making the diagnosis difficult to discern from myoclonus or other automatisms (Boylan et al., 2013; Mizrahi EM and Kellaway P, 1998).

The high incidence of electroclinical dissociation (ECD) of seizures in neonates may be related to the developmental profile and caudal-rostral pattern of maturation of the chloride cotransporters: NKCC1 and KCC2 (Dzhala et al., 2010; Glykys et al., 2009; Kahle and Staley, 2012; Sanchez and Jensen, 2001). The mechanism of electroclinical dissociation of seizures may be age-specific (Jensen, 2009a). The major inhibitor neurotransmitter gamma-aminobutyric acid (GABA) is immature in the developing neonatal brain and it matures only around the third or fourth week of life in rats (Brooks-Kayal et al., 2001), thus GABA is mainly excitatory in the early postnatal life (discussed in chapter 2). The depolarizing effects of GABA during early development combined with a delay in postsynaptic inhibitory systems causes seizures to be more easily elicited in the developing neonatal brain (Holmes and Ben-Ari, 2001).

Phenobarbitone is a GABA agonist and it has been shown to inhibit EEG seizures less effectively than clinical seizures; this causes phenobarbitone to exacerbate the dissociation of electrographic seizures (an incidence of up to 80% after use of anti-seizure medications (Scher et al., 2003)) from clinical seizures (Kahle and Staley, 2012). The hypothesis for this occurrence has been based on the ontogeny known as the KCC2 mRNA expression which follows a caudal-rostral pattern. The spinal cord and subcortical neurons begin to express KCC2 early during embryogenesis, while KCC2 expression in cortical neurons increases only after birth (Stein et al., 2004; Wang and Kriegstein, 2011). The different expression patterns of NKCC1 and KCC2 suggests that at birth (discussed in chapter 2), GABA should have a more inhibitory effect in spinal and subcortical neurons when compared to cortical neurons, but there is no

direct evidence for a differential effect of GABA on cortical versus subcortical structures.

Glykys et al. tested the hypotheses on the mechanisms of electroclinical dissociation and its exacerbation by phenobarbitone; and found that the neocortex and subcortical structures have different intracellular chloride concentrations during postnatal development using the genetically expressed chloride-sensitive dual wavelength fluorescent protein Clomeleon (Glykys et al., 2009). Their experiments demonstrated and hypothesized that:

1. The intracellular chloride concentration varies substantially between neighboring neurons in both the developing thalamus and neocortex, but that the average intracellular chloride concentration is significantly lower in thalamic than in cortical neurons.
2. Phenobarbitone was an effective anti-seizure medication in the thalamus but not in the neocortex; this was hypothesized as a result of a net inhibitory effect of GABA in the thalamus but an excitatory effect in the neocortex.
3. The combination of bumetanide and phenobarbitone is effective in decreasing epileptiform activity in the neocortex while it is not different from phenobarbitone alone in the thalamus.

Based on these results, it was explained that there is a caudal-rostral intracellular chloride concentration maturation in neonates which determines the neuronal responses to GABA and that the differences in intracellular chloride concentrations may contribute to the mechanism of electroclinical dissociation of seizures and the exacerbation of this dissociation by GABA-related anti-seizure medications (Glykys et al., 2009). Due to GABA development in the neonatal developing brain and selective inhibition in specific regions determined by chloride concentrations, phenobarbitone has also been hypothesized not to have the ability to control seizures arising from subcortical/ neocortex structures but could better control seizures arising from the cortical structures (thalamus, amygdala) (Glykys et al., 2009).

More cortical lesions have been observed in neonates with electroclinical seizures and more subcortical lesions were seen in neonates with electroclinical dissociation of seizures; suggesting that subcortical lesions may be involved in

the dissociated seizures. In ECD seizures, the clinical component preceded the electrical component to a significant extent when compared to electroclinical seizures; this reflects that the foci closer to the effector pathways than the cortex may result in the delay in the cortical electrical expression following clinical manifestation (Glykys et al., 2009).

Clinical aspects of electroclinical dissociation of seizures

Scher *et al.* reported the incidence of electroclinical dissociation of seizures after the use of phenobarbitone and phenytoin in their cohort of neonates (Scher et al., 2003). Although, more than 30% of neonates (with birth asphyxia, intracerebral haemorrhage and infections) were shown to experience very frequent seizures, the vast majority had no clear clinical signs (Malone et al., 2009; Murray et al., 2008). The figure of greater than 60% has been commonly reported in human neonates (Boylan et al., 1999; Scher et al., 2003), and as high as 80% (Clancy et al., 1988) of all EEG seizures has also been reported. Using continuous EEG monitoring after at least one occurrence of clinical seizure in neonates from corrected gestational age of 28 to 46 weeks (88% of neonates received at least one anti-seizure medication before EEG recording (Clancy and Legido, 1987)), Clancy and Legido showed that 79% of subsequent EEG seizures were clinically silent (Clancy et al., 1988). Ultimately, this results in a large proportion of neonates being undiagnosed and therefore, remained untreated (Silverstein and Jensen, 2007).

Contrastingly, Weiner *et al.* found that anti-seizure medication was not solely responsible for the electroclinical dissociation of seizures in their cohort study (Weiner et al., 1991). Based on this finding, they concluded that dissociated seizures were equally likely to occur before as well as following treatment with anti-seizure medication. However, their study may be biased, as clinicians were more inclined to treat neonates who had abnormal clinical movements in the electroclinical group. This study has found that only 16% of neonates displayed electroclinical dissociation of seizures (51 neonates with various aetiologies and with gestational ages of between 23 and 42 weeks); they conceded that this number may have been an underestimation because not all neonates in this study had prolonged and continuous multichannel video-EEG monitoring performed (Weiner et al., 1991). Possibly, there may be other factors which may influence the occurrence of ECD (discussed in Chapter 9: the Electroclinical dissociation study).

3.1.3 Clinical movements mimicking seizure-like activity but with no EEG correlate

In neonates, clinical seizures that do not correlate to electrographic discharges are rare (Biagioni et al., 1998). There are several mechanisms which have been proposed to explain these clinical seizure-like behaviours without scalp EEG correlate. Neonates may display paroxysmal behaviours such as those seen in benign sleep myoclonus (Kaddurah and Holmes, 2009; Maurer et al., 2010), jitteriness (Shuper et al., 1991), breath-holding spells (Fejerman, 2005) and hyperekplexia (Dreissen et al., 2012; Praveen et al., 2001). These events have been considered by many authors to be unrelated to seizures because they were not accompanied by any changes on the EEG. Volpe has suggested that nonepileptic events should be suspected if there is sensitivity to sensory stimulation (e.g. sound, movement or ambient temperature), suppressibility by gentle restraining or repositioning of the neonate and the lack of accompanying autonomic phenomena such as decreased in peripheral deoxygenation (Volpe JJ, 2008).

Abnormal clinical behaviours in neonates can be due to a phenomenon known as the “release phenomenon” of primitive brainstem and spinal motor pathways, which are normally inhibited by a functioning forebrain (Alfonso et al., 2000). The lack of this inhibition (e.g. in early normal stages of brain development or in some cases of severe brain injury) causes the release of abnormal physical movements that may be erroneously interpreted as clinical seizures by the observer. Some authors have hypothesized that EEG seizures without clinical correlates may be originating from deep foci of the brain, distant from the scalp electrodes (Mizrahi EM and Kellaway P, 1998; Weiner et al., 1991).

Based on an anecdotal report, seizures in a human neonate (term female infant with atelencephaly) have been shown to emanate from non-cortical structures such as the deep grey matter structures (Danner et al., 1985). This was based on findings on EEG monitoring for 3 hours using the 10-20 electrode placements on day 4 of life; 17 electrographic seizures were observed while the infant remained motionless. However, there were no neuro-pathological studies performed and the confirmation of an absent brain cortex was lacking. Most clinicians and researchers consider in-depth EEG recording to be inappropriate or unethically justified.

3.2 Using clinical recognition

Seizures in neonates can present variable clinical expressions and can be extremely inconspicuous (Malone et al., 2009; Murray et al., 2008). Direct visual inspection of neonatal behaviours is the actual method of seizure recognition currently employed in virtually all units where there is no video-EEG facility. Innocent tremulous clinical movements may be erroneously identified as seizures; many abnormal clinical movements are not found to be related to any specific epileptic mechanism (Dreissen et al., 2012; Kaddurah and Holmes, 2009; Maurer et al., 2010).

Likewise, only 34% of EEG seizures have been shown to be accompanied with overt clinical signs (Murray et al., 2008); this implies that clinical detection of seizures will lead to both over and under estimation of the true seizure burden. Nursing and medical personnel vary significantly in their ability to recognize suspicious behaviour contributing to both over-diagnosis and under-diagnosis of neonatal seizures (Malone et al., 2009; Murray et al., 2008). Unaided by EEG monitoring, bedside clinical detection may seriously underestimate the real number of seizures expressed by neonates.

3.3 Using the amplitude-integrated EEG (aEEG)

The amplitude-integrated EEG (aEEG) was first developed in the late 1960s as a means of monitoring the brain activity in adults undergoing surgery, suffering head trauma, or in a coma (Maynard et al., 1969). In the mid-1980s research groups in Sweden and the Netherlands began investigating its use in neonates. Currently, the aEEG is favoured by neonatologists for prolonged monitoring of the neonatal brain in most neonatal units (de Vries and Hellstrom-Westas, 2005; Shah et al., 2008; Shellhaas et al., 2007) (figure 3.2).

Figure 3.2 Brain monitoring in the neonates using the aEEG based on 3 electrode placement for 1 EEG channel.



The advantage of the aEEG is its immediate availability, ease of application (usually using 3 to 5 scalp leads) and interpretation by bedside nursing and medical personnel, including neonatologists (de Vries and Hellstrom-Westas, 2005; Hellstrom-Westas and Rosen, 2006). With the new aEEG machines Stellate EEG system (Natus Medical Inc, USA) and the Brainz aEEG monitor (Natus Medical Inc., USA), automated seizure detection algorithms from Gotman (Gotman et al., 1997a) and Navakatikyan (Navakatikyan et al., 2006) respectively have been incorporated in the machine and has been implemented routinely in the neonatal intensive care unit. The multichannel EEG differs from the aEEG in that it involves a larger numbers of electrodes (at least 10-20 electrodes). The multichannel EEG is considered labour-intensive in terms of the setup as well as in interpretation (Lawrence et al., 2009).

The aEEG is a compressed, filtered and processed form of the EEG. The aEEG gives information about trends over time in the amplitude (upper/lower) of the EEG. Current aEEG trend recording is simultaneously displayed with the original conventional raw EEG signal from the same recording channels; this allows artefacts (for example muscle activity and electrical interference) to be distinctively detected during the monitoring. The aEEG trend is, like the conventional EEG, mainly interpreted through visual pattern recognition, including assessment of continuity and discontinuity of cerebral activity, appearance of sleep-wake cycling (discussed in section 4.1) and indication, but not confirmation, of seizures.

The aEEG has a system which is designed to generate an output of lower amplitude signals ranging from 1 to 10 μV so as to capture a depressed cerebral activity (Hellstrom-Westas, 2008; Hellstrom-Westas and Rosen, 2006); hence the amplitude scale in the original cerebral function monitor was linear from 0 to 6 μV , a semi-logarithmic scale from 8 to 20 μV and logarithmic at $>25 \mu\text{V}$ using the Fourier spectral transform (Shah et al., 2008). The interpretation on the aEEG is based on a trend display, which shows a heavily time-compressed signal after it has been extensively filtered.

The aEEG is now commonly used to assess the EEG background in neonates and allows continuous assessment of long-term changes in cortical background activity (Boylan et al., 2010; Filan et al., 2007; Toet and Lemmers, 2009), and this can be done based on assessments of trends either by voltages or pattern recognition (figure 3.3 and 3.4). The very early aEEG background pattern has a very high predictive value in asphyxiated term infants (Spitzmiller et al., 2007).

Figure 3.3 Trends of aEEG traces and EEG showing the interpretation schemes based on voltages.

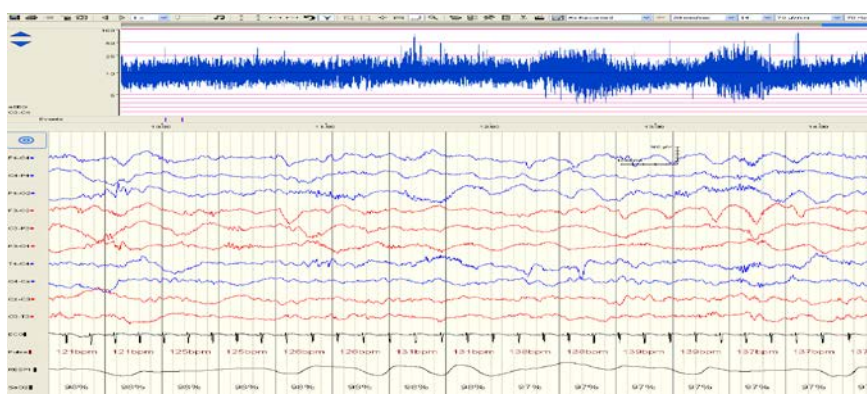
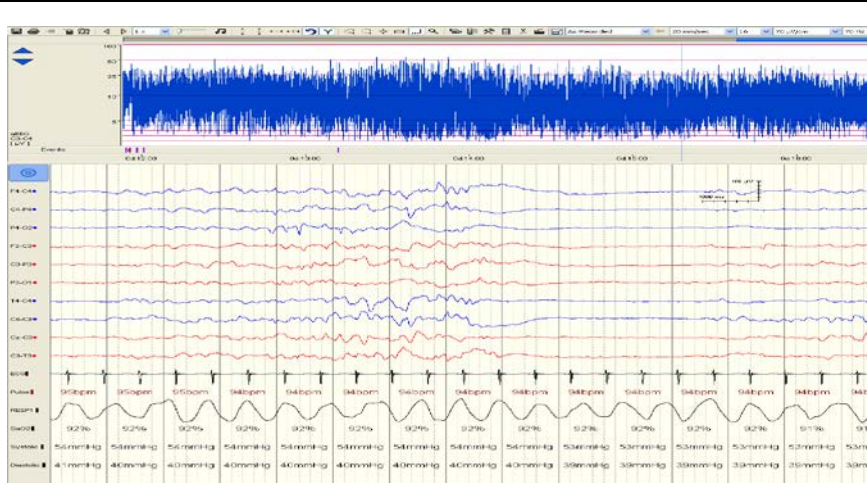
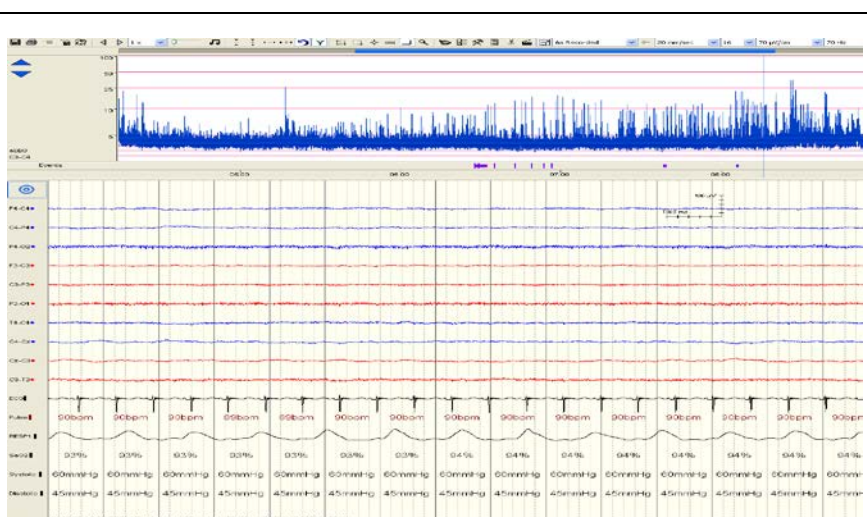
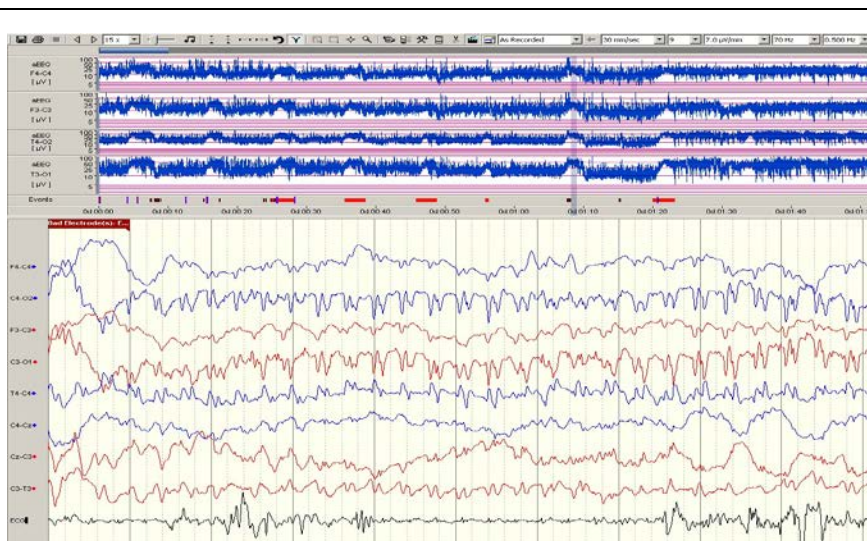
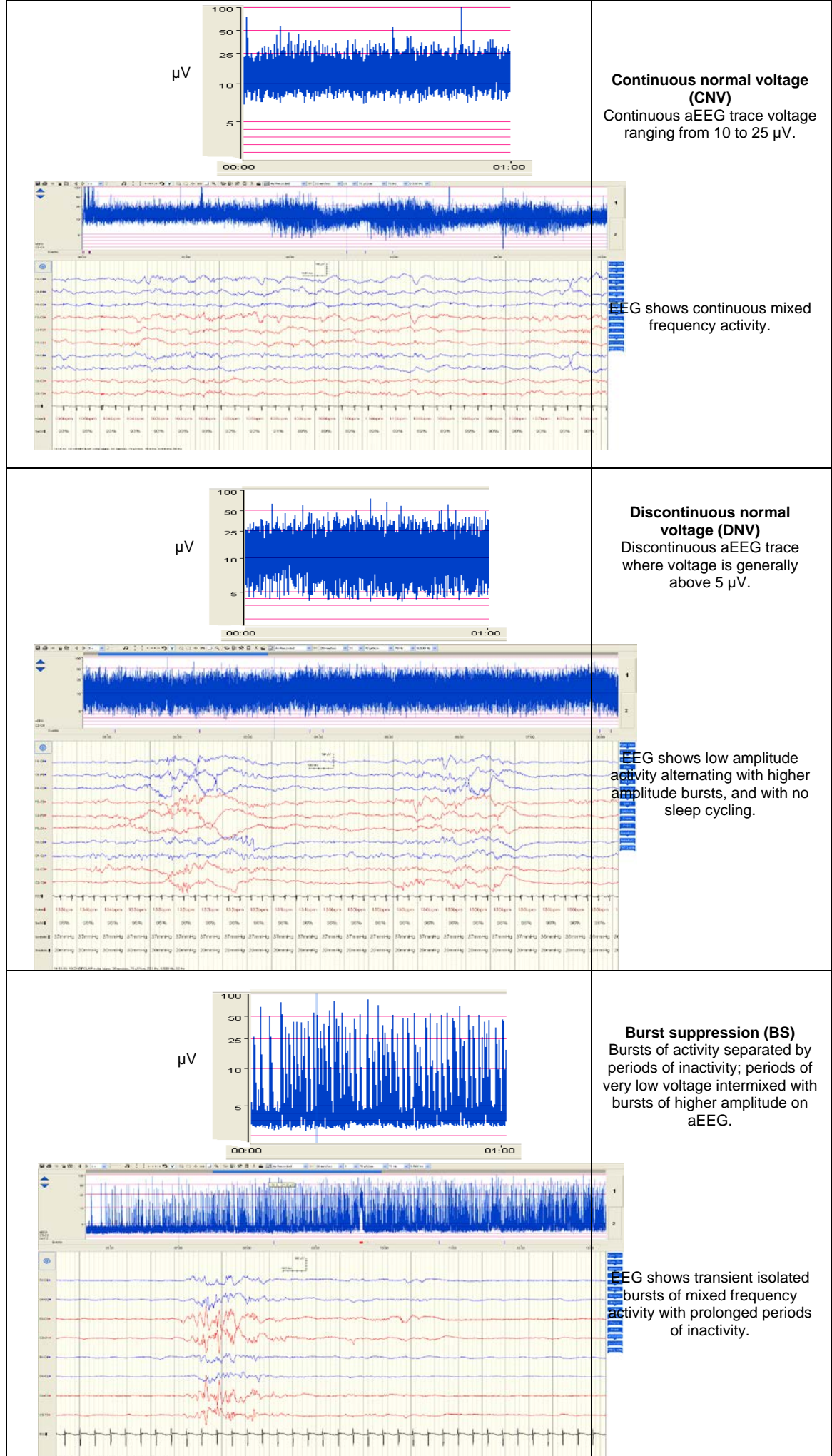
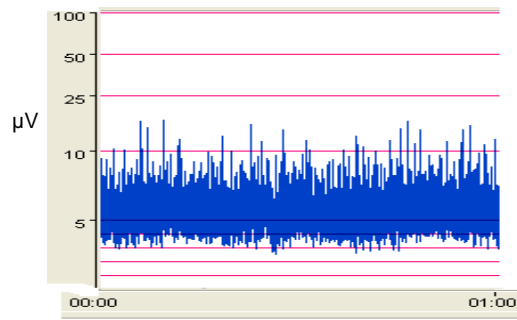
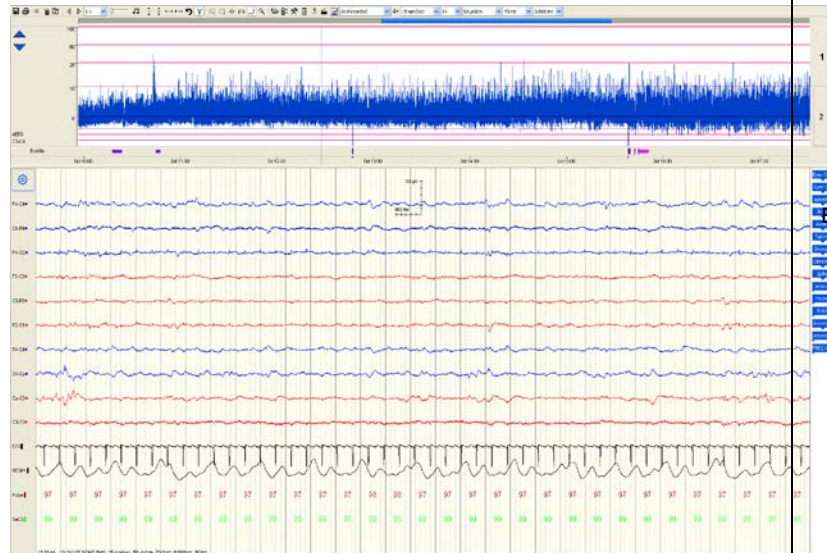
| | |
|--|--|
|  | <p>Normal aEEG</p> <p>The upper margin of the dense aEEG band is greater than 10 μV and the lower margin is greater than 5 μV.</p> <p>EEG shows continuous mixed frequency activity.</p> |
|  | <p>Moderately abnormal or discontinuous aEEG</p> <p>The upper margin of the dense aEEG band is greater than 10 μV and the lower margin is less than or equal to 5 μV.</p> <p>EEG shows discontinuity.</p> |
|  | <p>Severely abnormal aEEG</p> <p>The upper margin of the dense aEEG band is less than 10 μV and the lower margin is less than 5 μV.</p> <p>EEG show infrequent bursts of low amplitude activity.</p> |
|  | <p>Seizure on aEEG</p> <p>Saw-tooth pattern on the aEEG.</p> <p>Seizure on EEG</p> <p>EEG showing repetitive stereotyped waveforms with a definite beginning, middle and end.</p> |

Figure 3.4 Trends of aEEG traces here showing the interpretation schemes based on trends

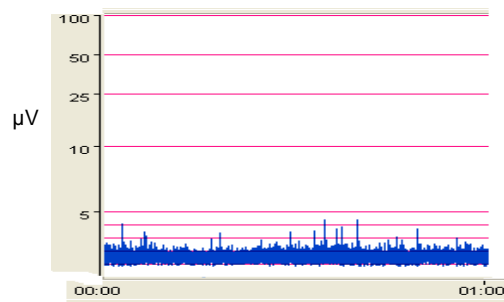




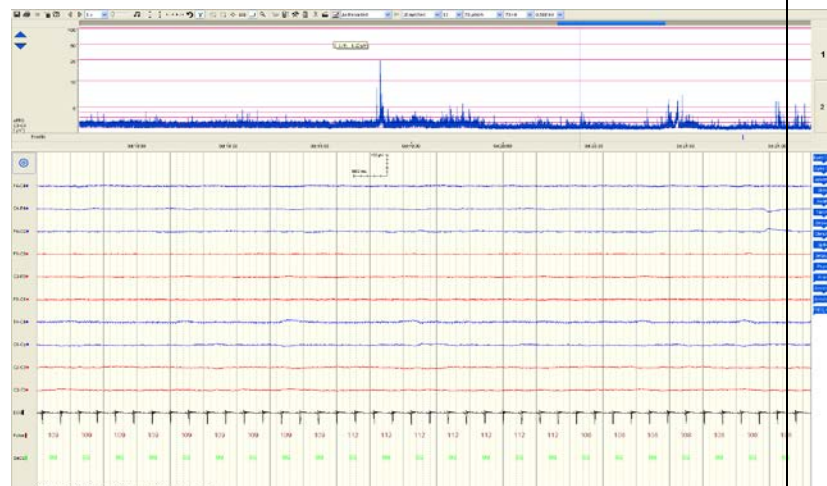
Continuous low voltage (CLV)
Voltage at or below 5 μ V.



EEG activities are continuous but low amplitude.



Flat trace (FT)
Virtual or complete inactivity; with voltages below 5 μ V.



EEG shows inactivity.

Historically, the aEEG signal is recorded from a biparietal derivation (P3-P4). The P3-P4 location was selected because it overlies the apex of the cerebrovascular watershed zone which is vulnerable to brain injury, and which borders a zone of arterial supply from 3 cerebral arteries (Hellstrom-Westas and Rosen, 2006), it has been shown to detect more seizures than frontal electrodes (Wusthoff et al., 2009). However, the adjacent C3 and C4 channels has been shown to provide comparable data for single-channel aEEG (Shellhaas and Clancy, 2007).

Although the aEEG has been successful in the early detection of severe disturbances in the background EEG activity (such as inactive or burst suppression traces) (Hellstrom-Westas and Rosen, 2006), the multichannel EEG is still deemed as a reliable for the detection of seizure in neonates as artefacts may be misinterpreted as seizures by non-experts using the aEEG (Rennie et al., 2004). The aEEG can be considered as a form of seizure detection algorithm as it displayed seizure activity by exhibiting a saw-tooth pattern. However, with restricted number of channels, this limits its use in locating seizures from other regions of the brain where the EEG electrodes are not placed.

Shellhaas and Clancy had used the central C3-C4 channel (C3 is approximately 4 cm anterior to P3 in term neonates) in neonates to create a single channel aEEG which was then used for comparison with the multichannel EEG (Shellhaas and Clancy, 2007). From a total of 125 infants (gestation 34 to 50 weeks), 851 seizures [mean duration: 32 (10-2314) seconds, mean seizure burden defined as the % of EEG recorded seizures at any location: 24.8 (0.7 to 86.9)%] were obtained from 125 conventional EEG (duration of monitoring 23 to 145 mins).

Shellhaas *et al.* then assessed the comparison of seizure detection among 6 neonatologists experienced in visualizing seizures on EEG or aEEG (Shellhaas et al., 2007). Although theoretically the seizure detection rate by the C3- C4 channel was 78%, the actual sensitivity based on visual analysis by 6 experienced neonatologists from 851 individual seizure detection was only 12 to 38% and 22 to 57% of the 125 conventional EEG records (Shellhaas et al., 2007). These studies from Shellhaas *et al.* showed that even among the most experienced neonatologists, seizure detection by visual analysis varied widely and are difficult to detect on the aEEG. However, it also proves that seizure detection by aEEG is better than visual analysis alone from neonatologists.

In contrast, Toet *et al.* reported a sensitivity of 80% for seizure detection based on 33 aEEG traces using the P3-P4 channel in 36 neonates with gestational age above 36 weeks (Toet et al., 2002). The differing reported rates of accuracy between most studies are difficult to interpret, as this may be related to the different single channel used in aEEG, the level of user experience, or that multiple seizure recordings from the same patient were studied without adjusting for clustering in the data (Glass et al., 2013; Rennie et al., 2004).

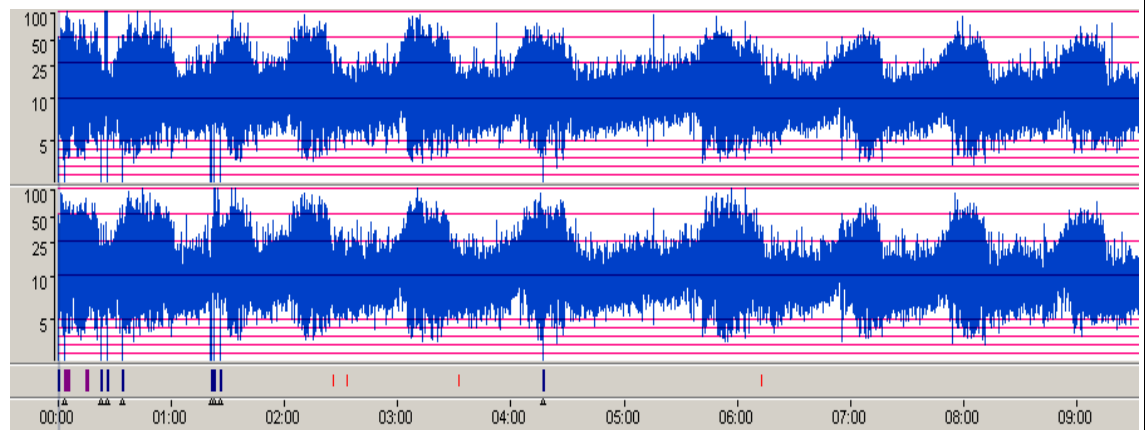
The weaknesses of using reduced montages in the aEEG are the risk of overestimating or underestimating the number of seizures. The aEEG has been shown to fail in detecting regional or focal seizures if the single channel recording is not adjacent to the brain region involved with the seizure expression (Scher, 2002). Seizure focus may not be exactly located under the recording electrodes, and neonatal seizures may present with many varying waveforms. Fewer than 3 of 10 neonates with suspected seizures on a single channel monitoring device had been shown to be verified by the multichannel EEG (Rennie et al., 2004).

Consistently, studies have shown that when using the aEEG from one single channel on a compressed timescale (figure 3.5), seizures with a duration of less than 30 seconds can be missed, as well as those which have a focal and of low voltage seizure activity (Rennie et al., 2004; Shellhaas and Clancy, 2007; Toet et al., 2002). Despite the inefficiency of the aEEG in detecting seizures, it is a device still being used worldwide for prolonged brain monitoring when the multichannel video-EEG recording is not available in the NICU. Sometimes, the aEEG is used as a complement to prolonged multichannel video-EEG recording. When seizures are suspected on the aEEG alone, experienced aEEG users have advised requesting multichannel video-EEG for confirmation of neonatal seizures (Hellstrom-Westas and Rosen, 2006).

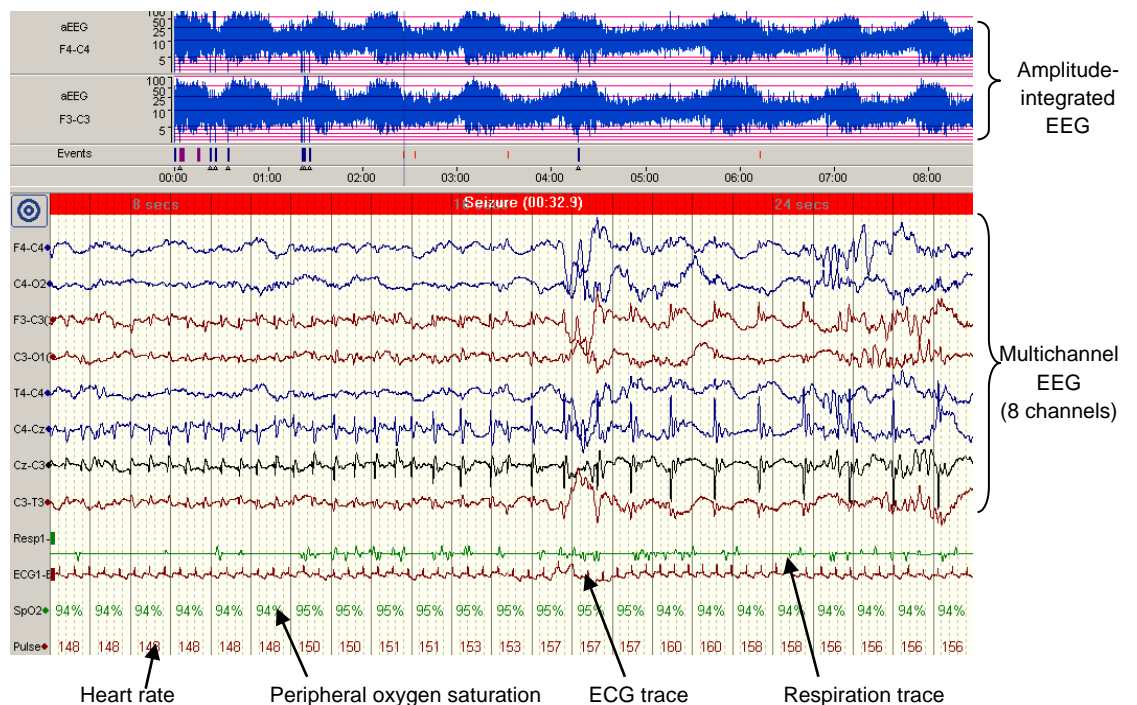
The clinical impact of the lower accuracy of aEEG in detecting seizures when compared with multichannel EEG is yet to be assessed. The effectiveness of the aEEG in detecting seizures remains debatable, principally when the interpretation solely rests in the hands of inexperienced or untrained users. Neonatologists must be attentive to the limited accuracy of aEEG in seizure detection in neonates. The use of clinical judgment, amalgamated with the use of the multichannel video-EEG monitoring is prudent when the diagnosis of seizures in neonates remains speculative.

Figure 3.5 An aEEG trace not picking up seizures consecutively shown on the multichannel EEG because seizures were of short in duration and of small amplitude.

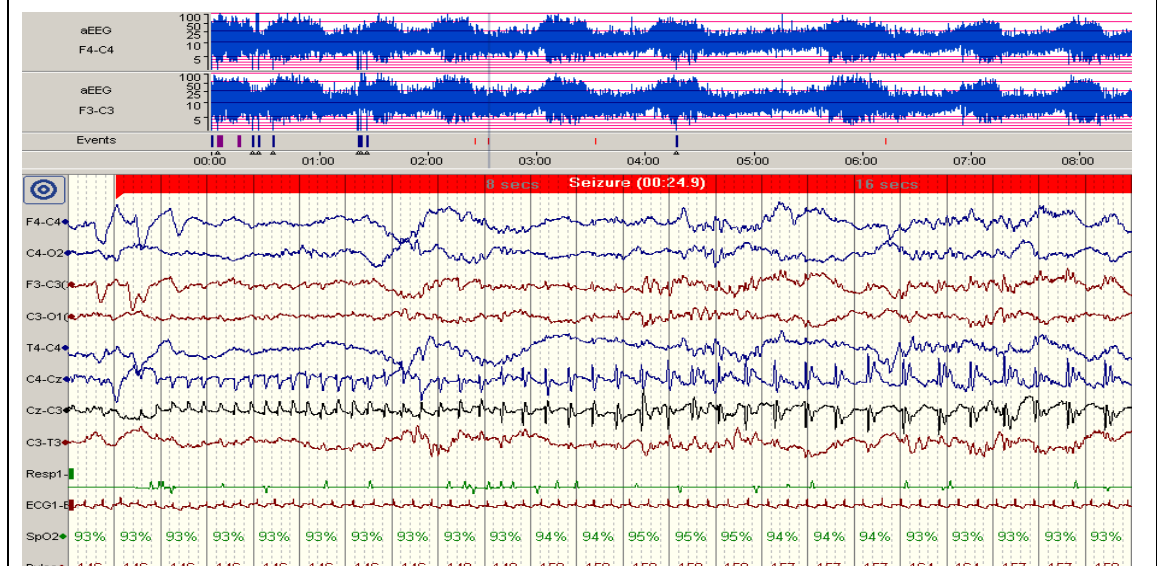
A full length of the aEEG compressed within a 9 hour period (electrographic seizures not detected by the aEEG trace as indicated by the red dots at the bottom of the trace)



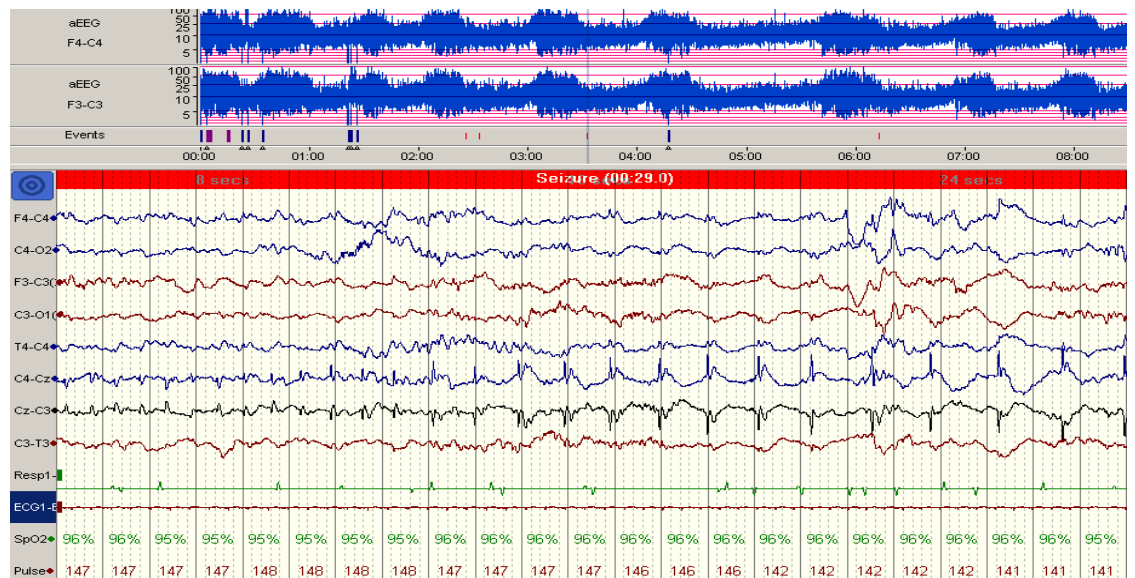
First electrographic seizure missed by the aEEG (highlighted by a faint vertical grey bar)



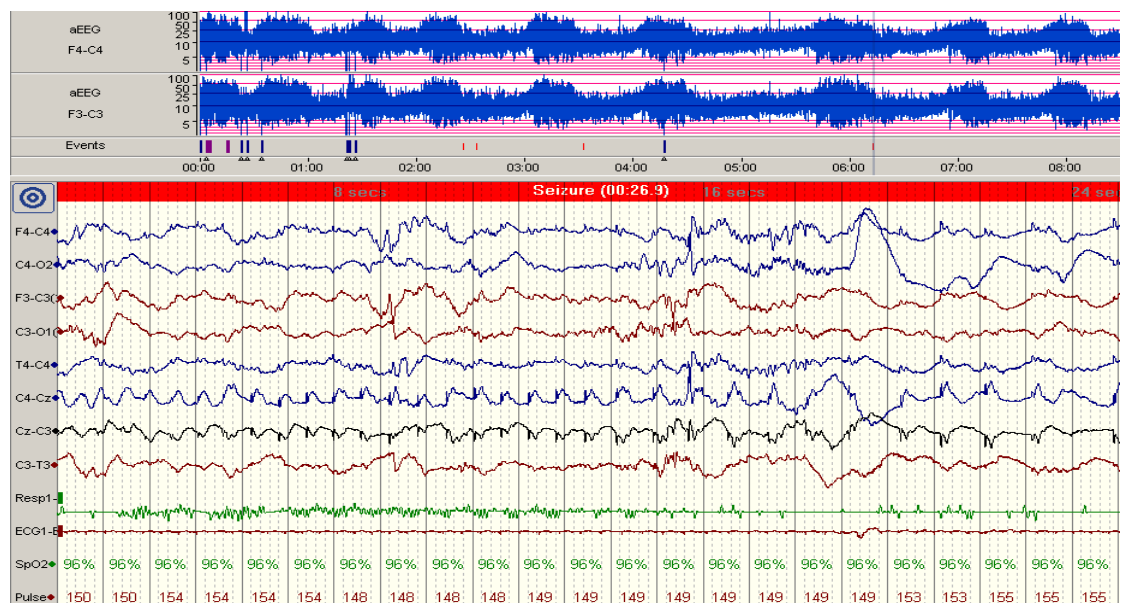
Second electrographic seizure missed by the aEEG



Third electrographic seizure missed by the aEEG



Fourth electrographic seizure missed by the aEEG



3.4 Using the multichannel video-EEG

Multichannel video-EEG is the most accurate tool for the identification and confirmation of neonatal seizures. Early and accurate detection of seizures is essential for guiding treatment with anti-seizure medication and in determining the risk of morbidity and mortality in neonates with brain injury (Boylan et al., 2010; Boylan et al., 2013; Boylan et al., 2015; Clancy, 2006a; Scher, 2002). The American Clinical Neurophysiology Society recommends either a full (16 electrodes) montage or a reduced (10 electrodes) montage for neonatal EEG monitoring (Klem et al., 1999).

To date, multichannel video-EEG, using the standard international 10 to 20 system modified for neonates (Klem et al., 1999), is the clinical gold standard for monitoring and recording seizures in the neonate. Unfortunately, the use of EEG

technology is often limited to specialized centers because the investigation must be performed by a specially trained technician, and proper interpretation requires a neurophysiologist who is familiar with the neonatal EEG. Furthermore in most centers, EEG access is limited during the evenings and weekends. These restrictions may limit the availability and/ or result in substantial delay between the exact moment when monitoring is needed and the exact moment when results are available before treatment. EEG monitoring is essential particularly during treatment of anti-seizure medication. Prolonged continuous multichannel and increased vigilance by the bedside has been shown to decrease cumulative EEG (Payne et al., 2014).

3.5 Using Neonatal Automated Seizure Detection Algorithm (NASDA)

One of the few limitations of multichannel EEG includes the availability of neonatal personnel for technical recording and interpretation. Interpretation of EEG findings could be immensely aided by EEG seizure detection algorithms. Since neonatal seizures are usually subclinical and are a potential risk factor for poor neurodevelopmental outcome warranting treatment as soon as possible, multichannel EEG monitoring with automated continuous and online seizure detection would be a very useful adjunct.

It is also needed because of the inherent limitations posed by the aEEG. Recent works on bedside monitoring have focused on automated detection of seizures (Boylan and Rennie, 2006; Cherian et al., 2011; Stevenson et al., 2013; Temko et al., 2011). A seizure detection rate by Gotman *et al.*, the automated system as high as 71% has been found (Gotman et al., 1997b). Another study has shown a sensitivity of 84% and a specificity of 98% (Cherian et al., 2011). Although the automated techniques hold the promise, the high false-negative rates posed by artefacts are the main limitation for current use as a screening tool for seizures in neonates.

Intermittent short EEG monitoring and sporadic interpretation in the neonatal intensive care unit has not been entirely useful (Clancy, 2006a; Shah et al., 2012). Although many studies have been conducted in relation to automated seizure detection in neonates, to date none of these automated seizure detection systems developed has been clinically utilized to process long EEG records in the neonatal intensive care unit (table 3.1).

Table 3.1 Comparison with other studies on automated seizure detection algorithm in neonates in terms of number of neonates, term or premature, the diagnosis in their cohort, training or validated dataset, EEG record and the number of seizures included in the study

| Author <i>et.al</i> | Number of neonates analysed | Term/ Preterm | Diagnosis | Dataset | EEG record (hours) | Seizure number (n) |
|--|-----------------------------|---------------|-----------------------------------|-----------------------|--------------------|--------------------|
| Liu 1992 (Liu et al., 1992) | 14 of 14 | ns | ns | Training | ns | ns |
| Gotman 1997 (Gotman et al., 1997a) | 54 of 54 | Mixture | ns | Training & Validation | 281 | 679 |
| Navakatikyan 2006 (Navakatikyan et al., 2006) | 17 of 55 | Mixture | ns | Training & Validation | 24 | 97 |
| Deburchgraeve 2008 (Deburchgraeve et al., 2008) | 21 of 26 | Term | HIE only | Training | 217 | 550 |
| Mitra 2009 (Mitra et al., 2009) | 28 of 48 | ns | ns | Training & Validation | 121 | 163 |
| Temko 2009-11 (Temko et al., 2009; Temko et al., 2011) | 17 of 17 | Term | Mixture (HIE, stroke, meningitis) | Training | 268 | 705 |
| Cherian 2011 (Cherian et al., 2011) | 24 of 24 | Mixture | Mixture (HIE, stroke, IVH) | Validation | 756 | 2077 |
| ns: not specified | | | | | | |

Few authors have attempted to develop a neonatal automated seizure detection algorithm based on different datasets as outlined in table 3.1. In 1982, Gotman *et al.* devised an approach to emulate the human observer in deriving values for duration and peak amplitude, slope of a series of consecutive waves for seizure detection (Gotman, 1982). From this study, a large percentage of seizures were correctly identified (81.42%) by EEG while generating a false positive rate of 5.38/h, mainly ascribed to artefacts such as electrocardiogram, electrode popping, eyes and muscle movements.

In 1997, Gotman *et al.* modified an adult seizure detection algorithm to detect neonatal seizure (Gotman et al., 1997a). Based on 3 methods (namely multiple spike detection, spectral analysis and detection of slow rhythmic discharges) applied to the EEG recording obtained from 55 neonates (8 to 16 channel EEG) from 3 hospitals, a total of 281 hours of recordings containing 679 seizures were analyzed by 1 electroencephalographer.

An initial evaluation indicated that 71% of seizures and 78% of seizure clusters (defined as a group of seizures separated by less than 90 seconds) were detected, with a reduced and improved false detection rate of 1.7/h (Gotman et al., 1997a). The lower false detection rate may be due to the exclusion of artefactual EEG segments in the pre-processing phase. In summary, Gotman *et al.* reported average seizure detection rates of 74% (Gotman et al., 1997a), 71% (Gotman et al., 1997a), and 69% (Gotman et al., 1997b) respectively in neonates with false detection rates of 2.4/h, 1.7/h, and 2.3/h. A reason proposed for the higher false detection rate in the study was that many “at risk” neonates had undergone cranial ultrasound imaging which involved prolonged manipulation of the head. Artefacts mimicking seizures on the EEG have been found to be related to events such as

gentle rocking, patting a distressed neonate, nursing or feeding the neonate (Stevenson et al., 2014).

The study by Cherian *et al.* described the sensitivity of a new seizure detection algorithm by testing a newer and larger EEG dataset on a previously improved algorithm called NeoGuard (Cherian et al., 2011). It is an automated neonatal seizure detection system which mimics a human interpreter with the visual scoring of the background EEG and with the automated seizure detection algorithm running on Matlab (The MathWorks, Natick, MA, USA) reading the EEG with the additional ability to reject artefacts. The dataset was from a cohort of neonates with hypoxic-ischaemic encephalopathy who were monitored by video-EEG for ≥ 24 hours from March 2003 to August 2007. Of 119 neonates, 45 had seizures. Twenty-one were excluded because they were already described in a previous article on seizure detection algorithm (Deburchgraeve et al., 2008). Three other neonates with stroke were excluded; the remaining 21 neonates formed their study group.

In the study by Cherian *et al.*, seizure amplitudes decreased considerably with deteriorating EEG background in 24 neonates (Cherian et al., 2011). Seizures were detected with a total sensitivity of 61.9% (1285/2077). The detected seizure burden was 66,244/ 97,574 seconds (67.9%). The average sensitivity in each neonate was 65.9% with a mean positive predictive value of 73.7%. After excluding four neonates with severely abnormal EEG background and who predominantly had dubious seizures (as defined in their study), the algorithm disclosed a median sensitivity per neonate of 86.9%, positive predictive value of 89.5% and false positive rate of 0.28/h. Sensitivity tended to be better for neonates with mild than moderate hypoxic-ischaemic encephalopathy.

Features such as the duration, amplitude and rhythmicity of electrographic seizures with regards to deteriorating EEG background, tend to worsen the performance of automated seizure detection. This article reported that the seizure burden in a group of neonates with severe abnormalities of EEG background activity was higher than in the mild to moderate group [3802 (880 to 15091) vs 1892 (127 to 6195) seconds; $p=0.16$]. Although all neonates in this study were said to have received a loading dose of phenobarbitone, the timing of phenobarbitone administration in relation to EEG commencement was not disclosed.

3.6 Conclusion

In both adult and neonates, seizures can have extremely variable morphology, frequency and topography, even within the same patient (Tekgul et al., 2005). Seizures in neonates are very different to seizures in adults and therefore require a specifically trained algorithm. The limitations of relying on clinical observation and the aEEG for neonatal seizure recognition are critical. It has led many to seek a better method of seizure detection at the cotside. The limited 24-hour service for reliable interpretation of the EEG by experts serves as one of the main deterrents for using multichannel EEG for many clinicians. Automated seizure detection may hold the key for prompt and more reliable seizure detection at the cotside. The method of seizure detection is crucial for the treatment of seizures. For treatment of seizures to work effectively, seizures have to be detected and monitored more reliably and at present this can only be done by performing prolonged and continuous multichannel EEG monitoring.

The Neonatal EEG and Electrographic Seizures

4.1 What is neonatal EEG?

The electroencephalogram (EEG) was first described in rabbits by Caton 1875 (Barlow, 1997). The measurement on human EEG was first reported in 1929 by the German psychiatrist Hans Berger (Barlow, 1997) (figure 4.1). In 1938, EEG recordings from term neonates were first published by Loomis *et al.* (Loomis AL et al., 1936) and Smith *et al.* (Smith JR, 1938). These early works have made a great contribution to our current knowledge of the neonatal EEG.

Figure 4.1 Professor Hans Berger (1873-1938)



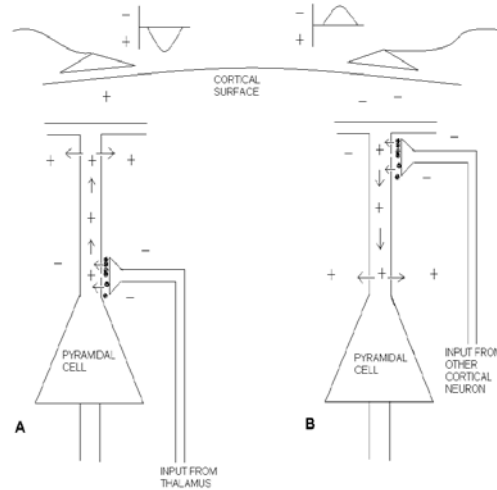
EEG recorded from surface electrodes is known to be the only window to assess the functional status of the cerebral cortex continuously and this is done by monitoring the electrical activity in the neonatal brain from the multiple scalp electrodes in real-time (Niedermeyer E and da Silva FL, 2004). Each electrode records from approximately a 2 cm patch of the underlying cortex to a depth of a few millimetres. This area contains millions of neurons, such that the EEG is recording the combined activity of these neurons. EEG waves are generated primarily from postsynaptic currents. The EEG measures the voltage fluctuations generated by summated excitatory and inhibitory postsynaptic potentials (figure 4.2 and 4.3). This electrical activity is a reflection of the summation of synchronous activity from millions of neurons that have similar spatial orientation.

The number of scalp electrodes used in neonatal EEG recording is reduced due to the small head circumference of the neonate. The minimum number of leads required are located at the frontal, temporal, central and occipital regions according to the standard international 10 to 20 system (Klem et al., 1999).

Figure 4.2 Origin of EGG (Niedermeyer E and da Silva FL, 2004).

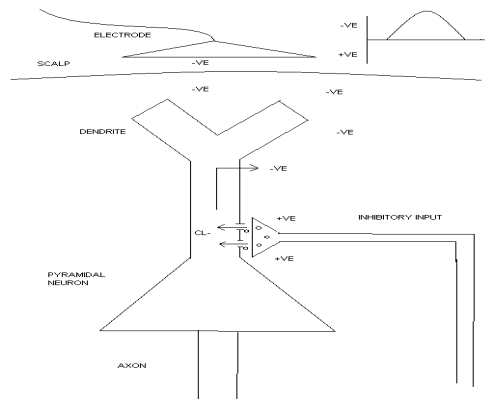
The polarity of the EEG depends on the net charge in the most superficial layer of the cortex.

A. Excitatory inputs



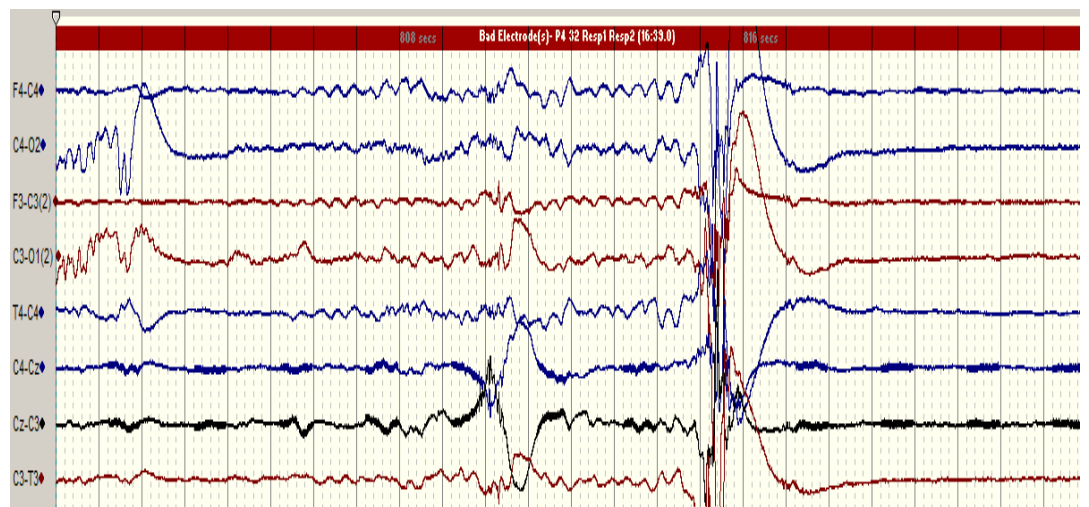
Excitatory inputs to cortical pyramidal cells causes sodium influx to the postsynaptic membrane and a local negativity in the extracellular zone around the synapse and a net positivity distal to it. Thalamic inputs synapse onto the dendrite proximal to the cell body creating a positive charged area at the surface. Inputs from other cortical neurons synapse distally to the cell body creating negative charged area at superficial layers.

B. Inhibitory inputs



Polarities are reversed for inhibitory inputs. Inhibitory inputs tend to synapse onto the proximal dendrite or cell body leaving a net negativity at the cortical surface.

Figure 4.3 Neonatal EEG

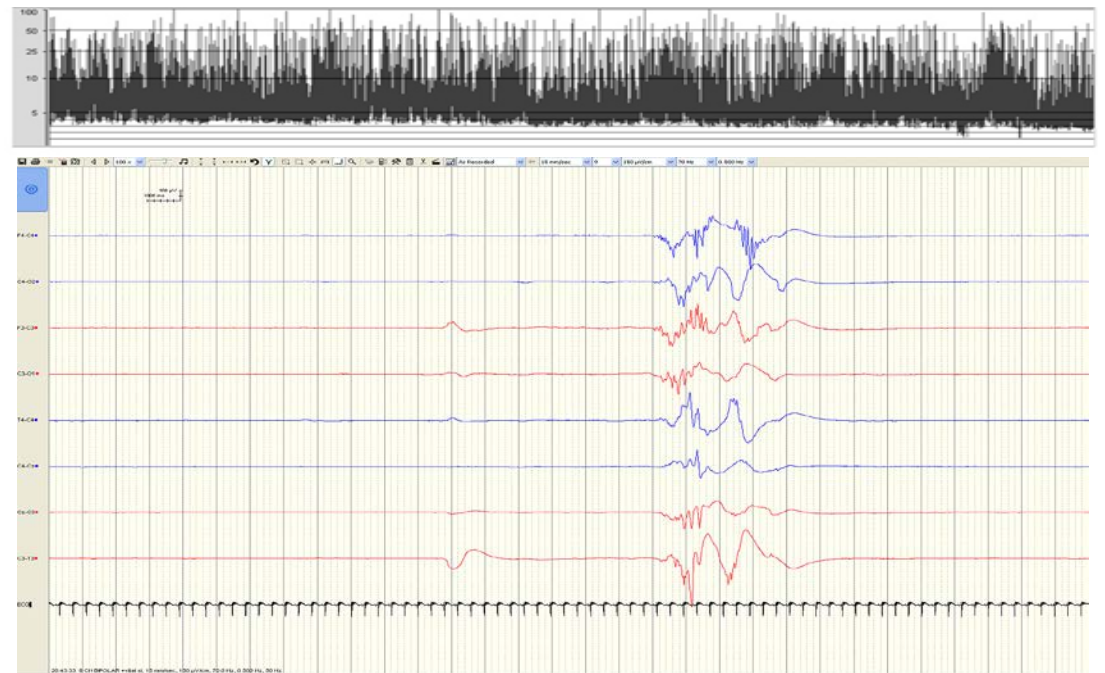


Multichannel EEG record showing 8 EEG channels of brain electrical activity from the central (C), frontal (F), occipital (O); parietal (P) and temporal (T) areas of the neonatal brain. There is a period of quiescence (horizontal lines) followed by a period of activity (sinusoidal lines) reflecting a normal brain activity. The bipolar vs. referential montage was used.

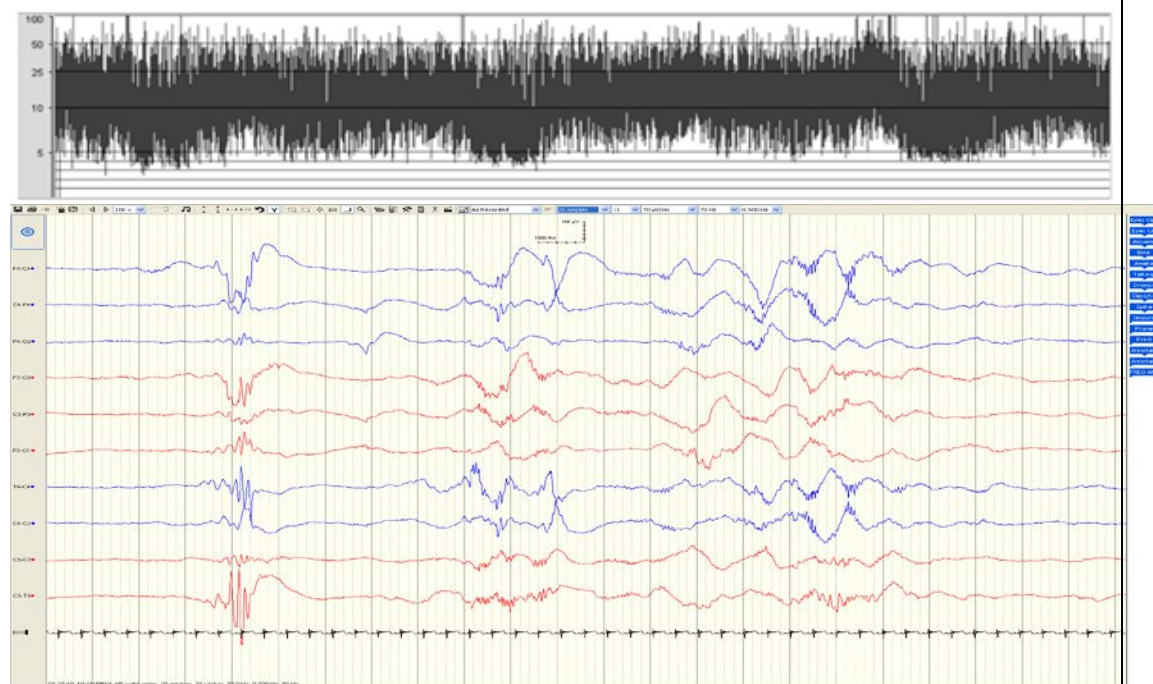
The amplitude of EEG differs with age: at term (beyond 37 weeks gestation) it is usually below 100 μV and preterm (below 34 weeks gestation) is usually below 300 μV (figure 4.4). The EEG in the earliest viable preterm neonates at 24 weeks is discontinuous; with bursts of transient activity with flattening of the EEG in between, and with interburst intervals of less than 60 seconds (table 4.1). As gestational age increases, the intervals between the bursts become shorter and lower amplitude activities replace the 'flattened' periods. By term gestational age, the EEG should be continuous.

Figure 4.4 The normal neonatal aEEG and EEG based on gestational ages

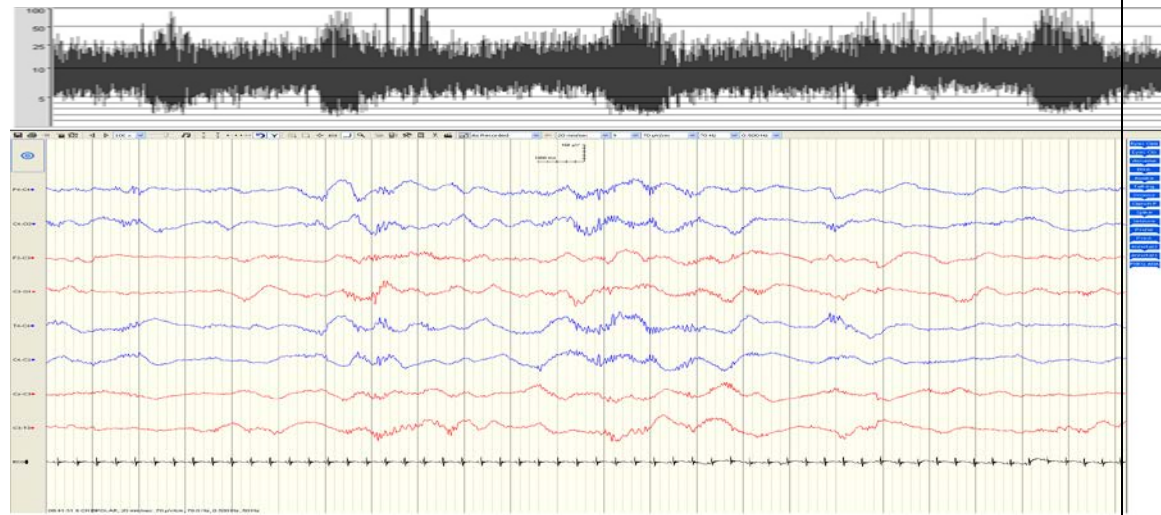
A. aEEG and EEG at 23 weeks gestation



B. aEEG and EEG at 30 weeks gestation



C. aEEG and EEG at 34 weeks gestation



D. aEEG and EEG at term gestation

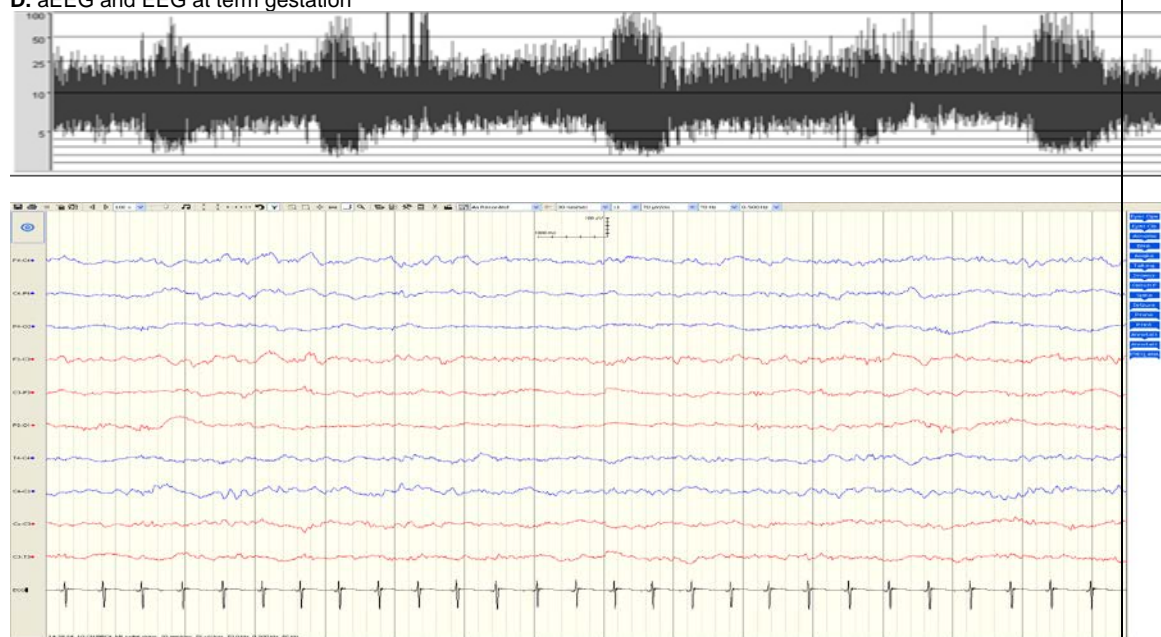


Table 4.1 Interburst intervals according to gestational ages (adapted from Rennie JM et al., 2008)

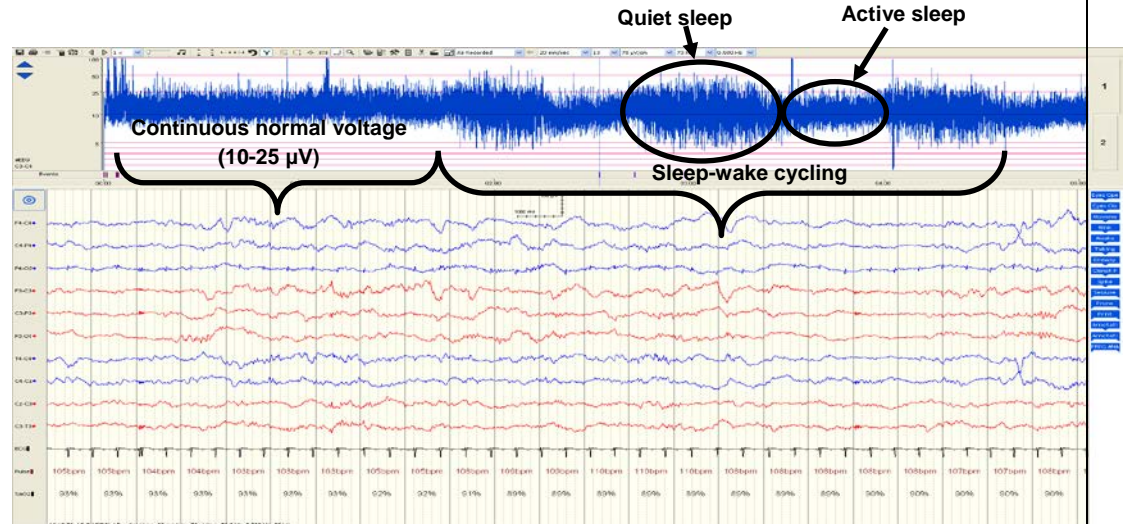
| Gestational ages (weeks) | Inter-burst interval (seconds) |
|--------------------------|--------------------------------|
| 24 to 26 | 10 to 60 |
| 27 to 29 | 10 to 40 |
| 30 to 32 | 3 to 20 |
| 33 to 34 | Less than 10 |
| 35 to 37 | Less than 10 |
| Beyond 37 weeks | None |

One of the usefulness of the neonatal EEG is to determine the presence of sleep-wake cycling in a neonate (Lamblin et al., 2013). Sleep cycles start to appear by 31 weeks and are clearly definable by 34 weeks. At term equivalent postmenstrual age, it is possible to differentiate between five conscious states of the newborn infant: wakefulness, drowsiness, active sleep (active moyenne, rapid eye movement sleep), quiet sleep (trace alternant, non-rapid eye movement sleep), and indeterminate sleep (slow wave sleep) (figure 4.5). By term gestational age, a

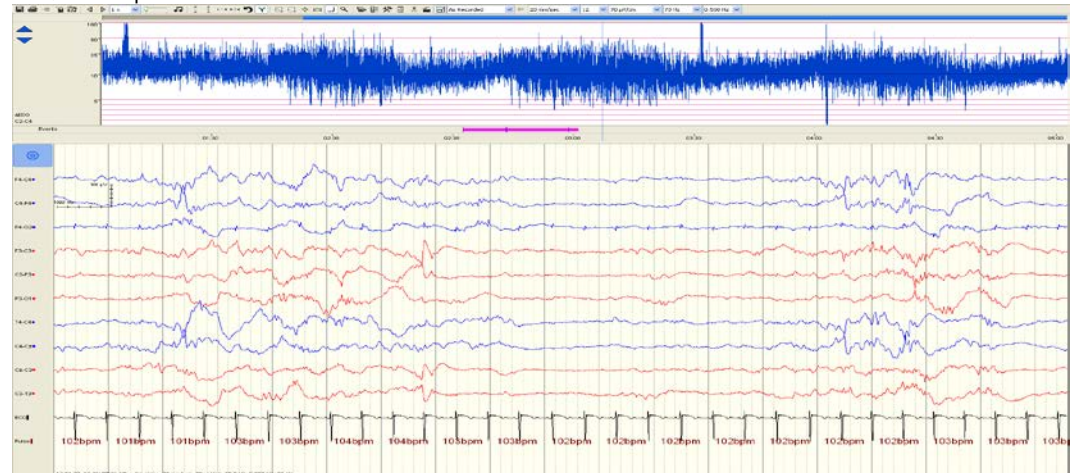
neonate will have full sleep cycle including quiet and active sleep in 1 hour. The standard eight-channel EEG enables identification of four different patterns of EEG background activity in one sleep cycle in healthy term infants: low-voltage irregular, mixed, high-voltage slow and trace alternant (Hellström-Westas L and Ingmar R, 2003).

Figure 4.5 Sleep-wake cycling

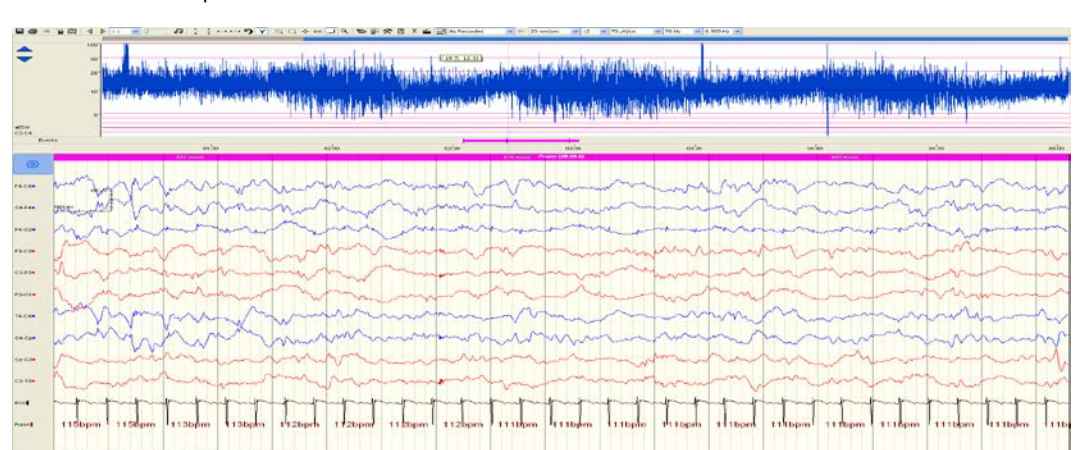
A. EEG shows continuous mixed frequency activity.

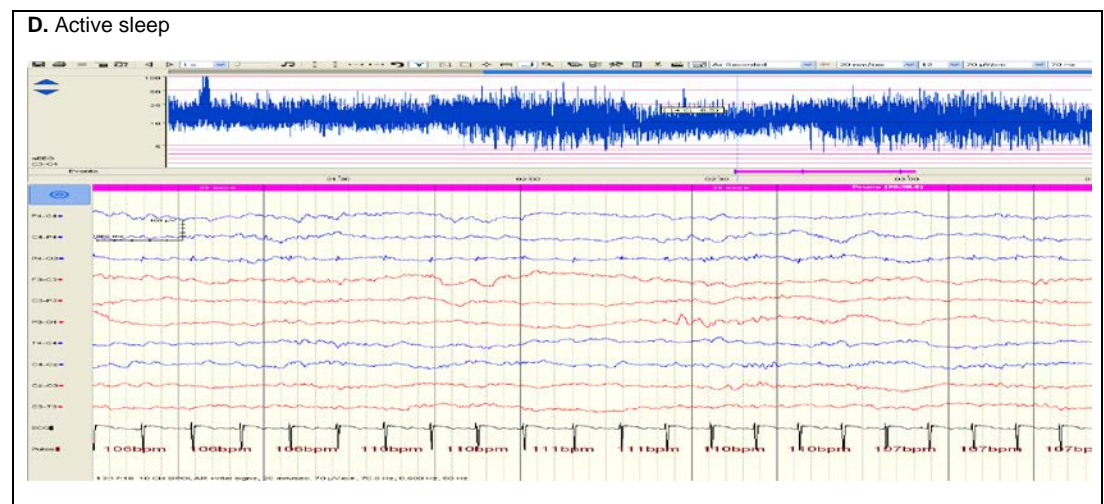


B. Quiet sleep. Trace alternant



C. Intermediate sleep

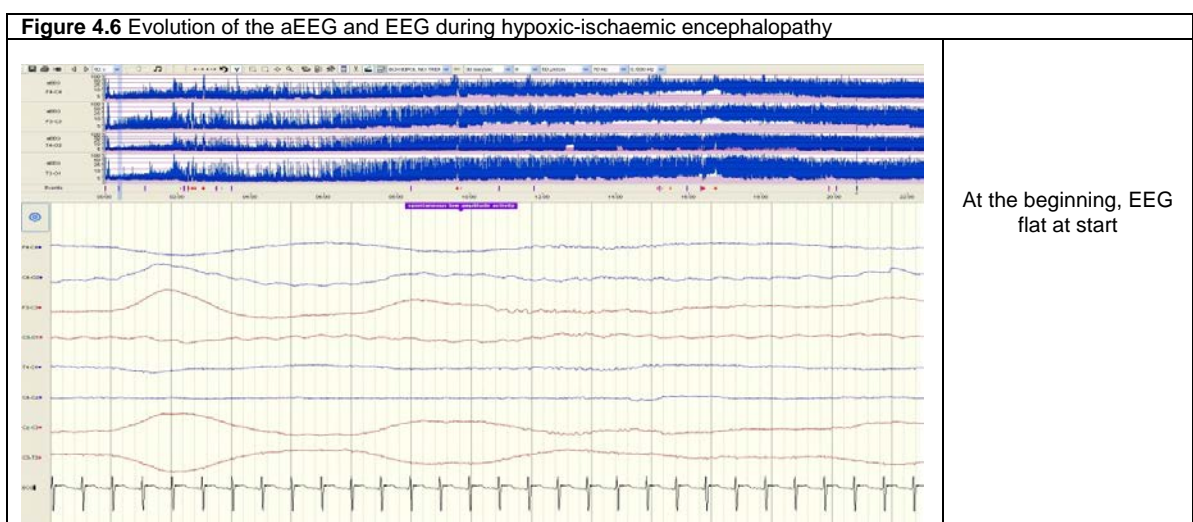


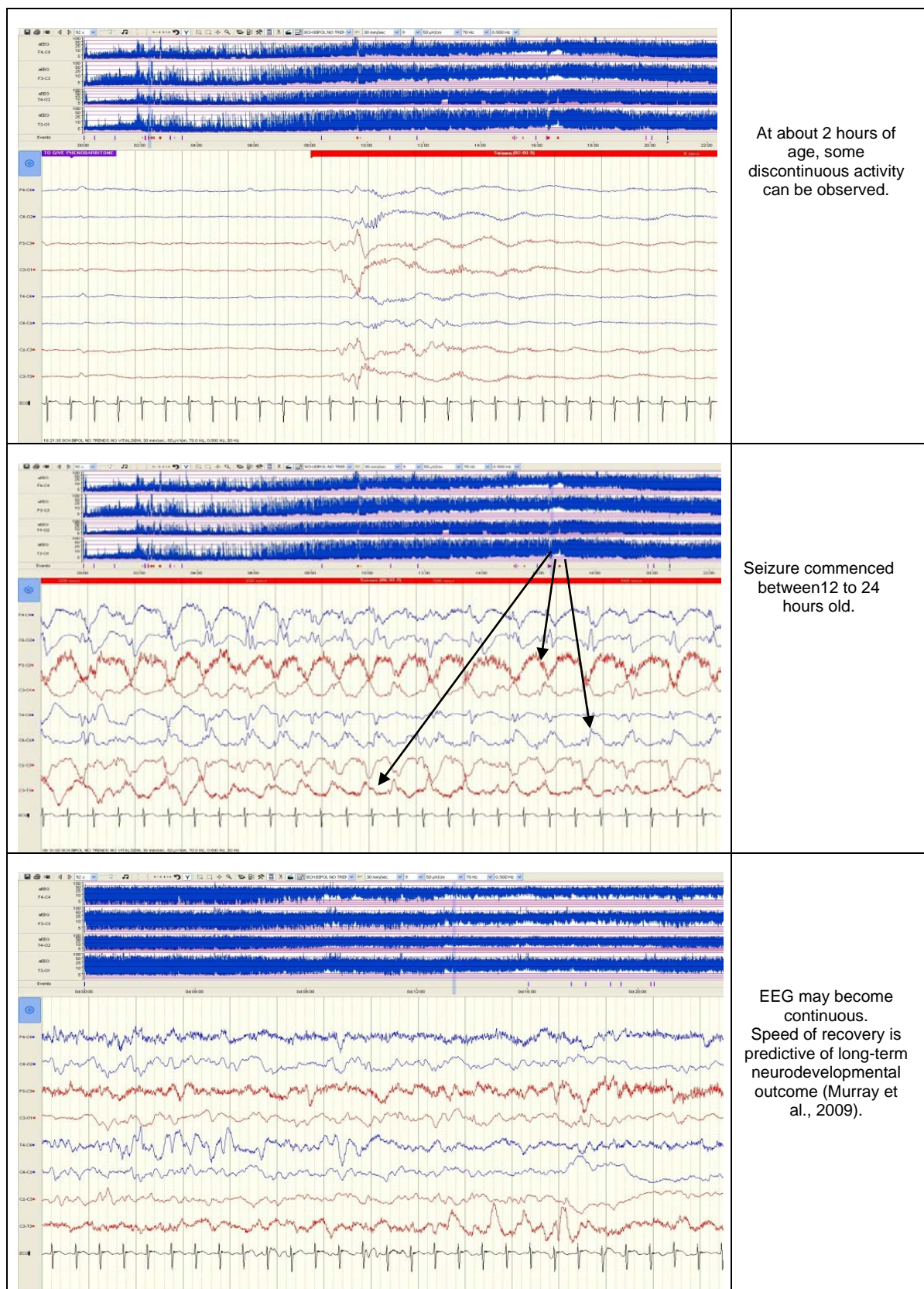


One cycle of four EEG patterns is thought to require 40 to 60 min, and on the aEEG, quiet/active cycling is also known as sleep/wake cycle. The trace is narrow when infant is awake (or in active sleep) and widens during quiet sleep; it typically cycles every 90 min, but is dependent on the condition but the neonate (Hellström-Westas L and Ingmar R, 2003). Low-voltage irregular and mixed activities are often observed during active sleep, whereas high-voltage slow and trace alternant activities are almost always observed during quiet sleep when sleep states are defined based on physiological parameters. This simplified scheme is not always fully consistent in practice because normal sleep cycle is easily disrupted and altered by external stimuli.

EEG in hypoxic-ischaemic encephalopathy

In regards to the EEG, following the primary hypoxia-ischaemic insult (discussed in chapter 2), due to the disruption to cerebral oxidative metabolism, cytotoxic oedema occurs and excitotoxins accumulate and the EEG becomes suppressed (figure 4.6). Some metabolic recovery is possible over the subsequent 30 to 60 minutes (Bennet et al., 2007; Tan et al., 1996).





A latent phase, which follows from about 1 to 6 hours, is characterized by cerebral hypoperfusion, reduced metabolism and a suppressed EEG. During this period, high energy phosphates can return to near normal values (Robertson et al., 2013). However, during the secondary injury phase which corresponds to further periods of cytotoxic oedema, accumulation of excitotoxins and hyperperfusion, there is a failure of cerebral mitochondrial activity which eventually leads to cell death.

(Bennet et al., 2006; Lorek et al., 1994; Wassink et al., 2014). In moderate to severe brain injury, the background EEG may start to recover during this period and seizures often develop. In very severe injury, the EEG can remain suppressed for days and seizures may never emerge.

4.2 Literature search on the definition of electrographic seizures in neonates

The most cited definition for electrographic seizures in neonates in the literature has been derived since 1987 by Clancy (Clancy et al., 1988; Clancy, 2006a); electrographic seizure was defined as the evolution of sudden, repetitive, stereotyped waveforms with a definite beginning, middle and end and with a minimal duration of 10 seconds. It remains a debate as to whether we should be treating single or short duration of electrographic seizures. Exclusively short electrographic seizures have been shown to possess similar poor prognostic value as longer electrographic seizure (Oliveira et al., 2000). In animal models, mainly longer and recurrent seizures such as status epilepticus have been shown to be more deleterious to the developing neonatal brain (Abend and Wusthoff, 2012; Topjian et al., 2013).

It is important to describe and define what electrographic seizures are in neonates before any estimation or quantification is pursued to establish the more precise seizure burden. This is vital for confirmation and annotation of electrographic seizures; both of which are still officially being performed through manual visual observation of long EEG recordings by independent neurophysiology experts.

Fundamentally, the definition of seizure (and subsequently the quantification of seizure burden) is dependent on whether the amplitude-EEG (aEEG) or the full multichannel EEG was utilised, the number of EEG channels used and the entire duration of EEG monitoring. In terms of seizure burden, several factors should be taken into consideration such as the gestational age, aetiologies and whether treatment with anti-seizure medication has been instigated in the study cohort before EEG monitoring begun.

4.2.1 Morphological features of electrographic seizures in neonates

4.2.1.1 Waveform patterns and frequency

The characteristics of electrographic seizures in neonates differ from those of adults in relation to clinical symptoms and pathology (Patrizi et al., 2003). It is common for electrographic seizures to first appear at relatively low voltage and to

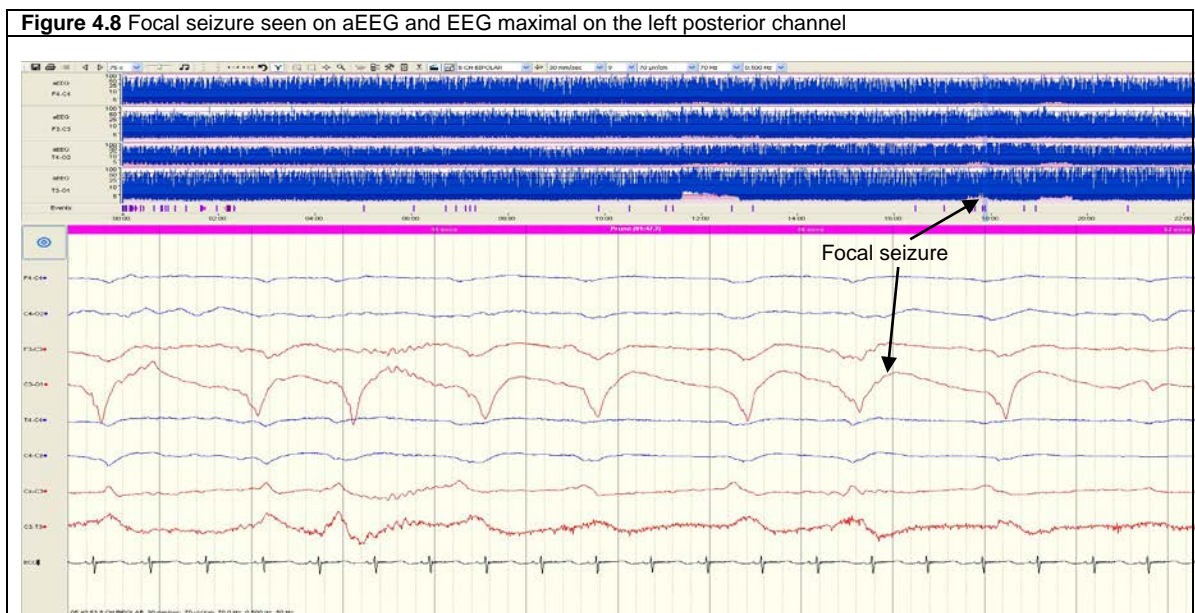
gradually increase as the seizure evolves (Patrizi et al., 2003). Neonatal electrographic seizures, not only have the ability to display a repetitive discharges of sharp waves with gradual changes in voltage and frequency; they also have the capacity to exhibit other characteristic features (Oliveira et al., 2000). One of these characteristics is an abrupt beginning and end of electrical discharges without any changes in character electrographically along the seizure (Oliveira et al., 2000); such that an electrographic seizure is often defined as a discrete event that has a definable beginning, middle and end (Clancy, 2006a) (figure 4.7).



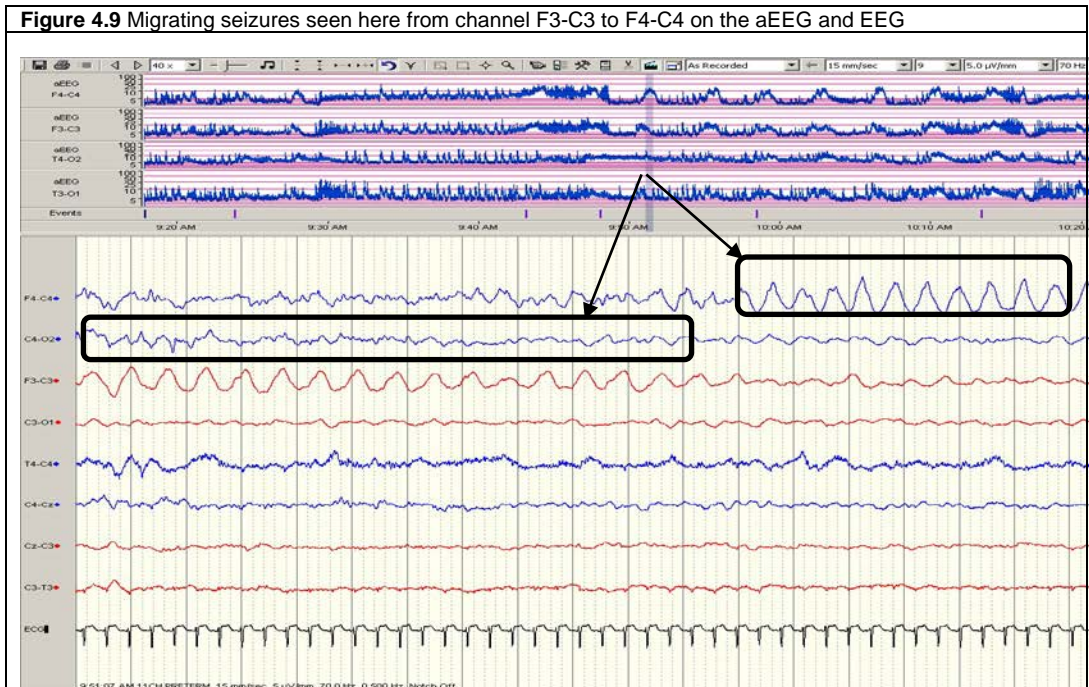
Seizures can be as a discrete event that has a definable beginning, middle and end (Clancy, 2006a) characterised in terms of their amplitude, frequency, morphology, location and duration. Nunes *et al.* observed that not only do neonatal seizure discharges originate focally, they can present as typical EEG waveforms such as trains of sharp waves, pseudo-beta-alpha-theta-delta patterns or multifocal discharges (Nunes and da Costa, 2010). At the onset of seizures, Patrizi *et al.* noted that preterm neonates predominantly displayed rhythmic alpha and delta activity, while term neonates predominantly exhibited sharp waves, spikes or slow waves (isolated or in combination) (Patrizi et al., 2003). The changing morphology of the discharges may be the result of a slow recruitment of additional neuronal networks during seizures.

4.2.1.2 Onset nature of neonatal seizures

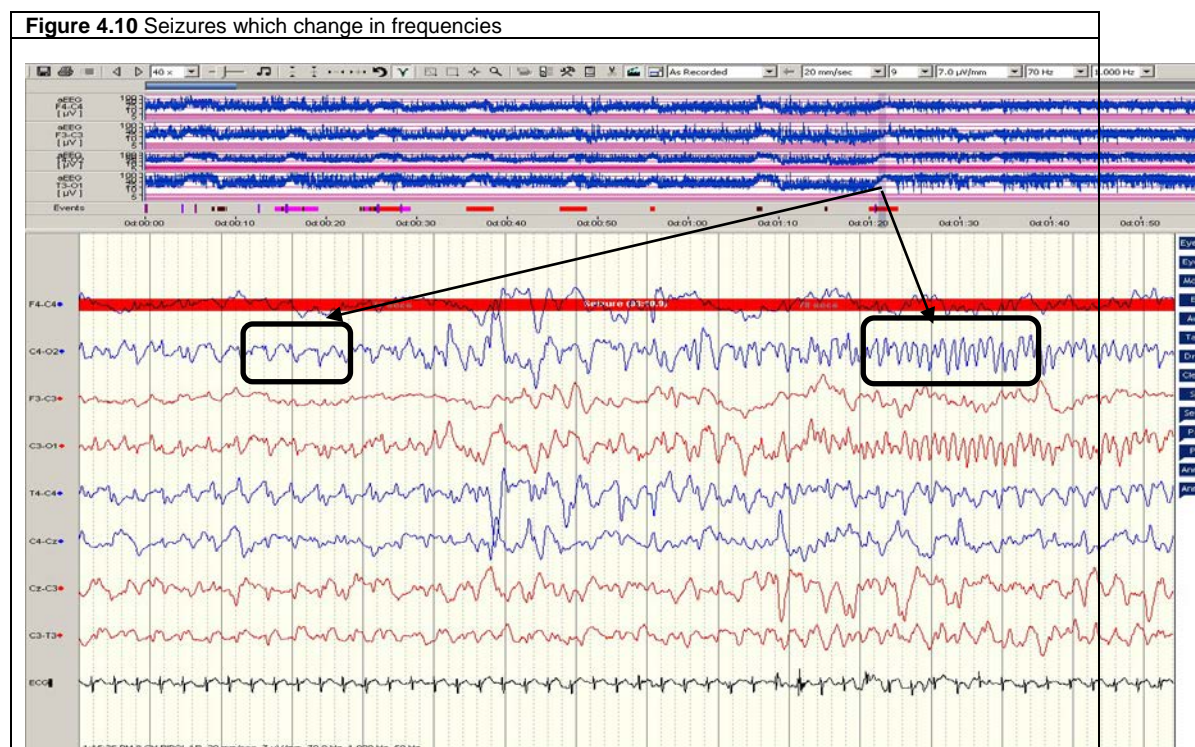
The term “focal” implies that the electrographic seizures are well localized to a small focus or region of the brain (Clancy, 1996). The fundamental observation is that neonatal electrographic seizures essentially arise focally (figure 4.8). For this reason, neonatal seizures have been described as partial seizures (Clancy, 1996). Neonatal seizures are topographically restricted (Clancy, 2006a; Tekgul et al., 2005) due to the characteristic focal nature of seizure onset. Therefore, seizures may be missed when using a limited number of electrodes.



The term “unifocal” is used, if the EEG or electrographic seizures originate from the same single location (Clancy, 1996). If repeated electrographic seizures always arise from the same single location, then it may suggest that a focal or lateralized structural cerebral lesion exists. Neonatal stroke is an example where repetitive seizures often arise focally, such that all of the individual electrographic seizures have their origin at the same location (Clancy et al., 1985). However, a single focal seizure does not necessarily convey the location of a restricted brain lesion or abnormality, and it can also migrate (figure 4.9). Patrizi *et al.* has shown that the focal nature of EEG or electrographic seizures was confined most commonly in term than in preterm neonates (Patrizi et al., 2003).



The term “multifocal” seizures mean seizures can originate from a number of locations. The onset of electrographic seizures arising from various locations of the brain were noted in 44% in a group of term and preterm neonates (Bye and Flanagan, 1995b). Multifocal seizures may appear in both hemispheres and progress independently at different frequencies (figure 4.10), often requiring at least 3 independent generators of seizures involving both hemispheres; they can also be expressed independently in anatomically unrelated brain regions (Volpe JJ, 2008).



4.2.1.3 Origin of location of neonatal seizures

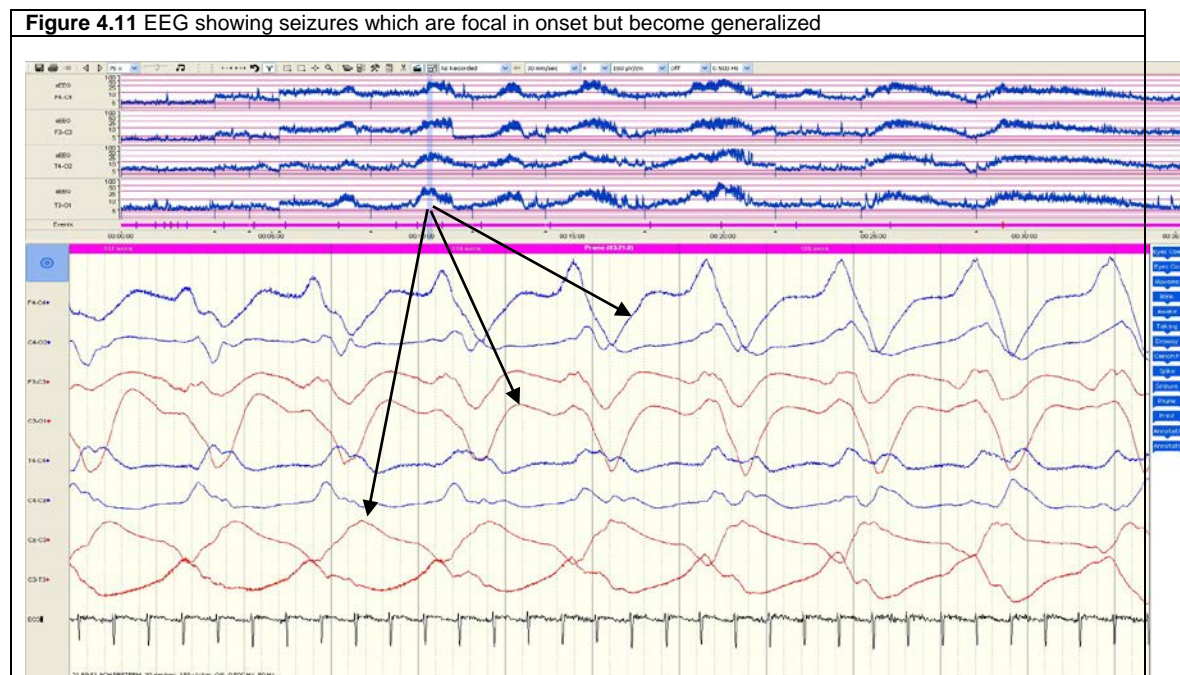
There are controversies as to which region of the developing neonatal brain has the predilection to generate seizures. In both preterm and term neonates, it has been hypothesized that the most common site of electrographic seizure origin is the temporal lobe (Patrizi et al., 2003). Within the temporal region, the mid-temporal (T3 and T4) are probably the most common locations of origin of neonatal EEG seizures, although the exact origin of individual neonatal EEG seizure may vary within an individual (Clancy, 1996). However, this has not been related to aetiology, but has been related to the combination of high cell density, intrinsic bursting cells and extensive recurrent excitatory collaterals which render the hippocampus in the temporal region to be highly epileptogenic in neonates (Holmes, 1997; Moshe, 1987). This is in contradiction to the rationale for placing electrodes during aEEG monitoring in the biparietal (P3 and P4) region which overlies the apex of the cerebrovascular watershed zone; an area which has been postulated to be the most vulnerable site for injury and borders a zone of arterial supply from the 3 cerebral arteries.

Since the bi-parietal (P3 and P4) region is not included as a prerequisite in the standard international 10 to 20 system of electrode placement for neonatal EEG monitoring, the more common central (C3 and C4) region electrode placement is used (C3 is 4 cm anterior to P3 in term neonates). Further studies are required to ascertain and confirm which electrode placements are most appropriate, in order to correctly capture seizures origin in the developing neonatal brain.

4.3 Status epilepticus in neonates

The incidence of status epilepticus varies from author to author due to different definitions applied to neonatal seizures (Lawrence and Inder, 2010; McBride et al., 2000; Scher, 2002; Wusthoff, 2013). Human studies have shown that the duration of seizures during status epilepticus are directly related to morbidity and mortality (Agarwal and Fox, 2013; DeLorenzo et al., 2009; van der Heide et al., 2012). The commonest definition cited by many authors today for neonatal status epilepticus stemmed mainly from a study published in 1993 by Scher *et al.*, which defines status epilepticus as a continuous electrographic seizure lasting for at least 30 minutes, or more than or equal to 50% of the EEG recording time, or the combination of both (Scher et al., 1993).

However, EEG recording times may vary considerably; traditionally as short as 1 hour was standard, currently it can last at least 72 hours (with the advent of therapeutic hypothermia). Because of the prevalent use of the aEEG among neonatologists worldwide, status epilepticus was defined as a saw-tooth pattern on the amplitude-integrated EEG (aEEG) lasting at least longer than 1 hour (de Vries and Toet, 2006; Toet et al., 2008; van Rooij et al., 2010a) (figure 4.11). Yet to date, there is no consensus on the definition for neonatal status epilepticus, despite the proposed criteria (Wusthoff, 2013).



Experimentally, ongoing seizures lasting for at least 30 minutes have been shown to cause neuronal injury in neonatal animal models with ischaemia (Fujikawa, 2005; Klitgaard et al., 2002); this forms the rationale for the definition of neonatal status epilepticus. The mechanism for brain injury secondary to status epilepticus has been attributed to toxic amounts of glutamate (Saghyan et al., 2010; Zhang et al., 2004). Glutamate leads to excessive depolarization of neurons, which results in intracellular increases of sodium and calcium. Changes in these intracellular ions lead to a cascade of events ultimately resulting in cell death (Fujikawa, 2005; Zhang et al., 2004). Prolonged seizure duration is thought to potentiate the risk of permanent brain damage and increases the difficulty of stopping seizure activity (Ben-Ari, 2006; Holmes and Ben-Ari, 2003; Lado et al., 2002), thus generating the belief that the best chance of terminating a seizure is with early treatment (Abend and Wusthoff, 2012).

Morbidity due to status epilepticus appears to be decreasing; this may be related to the improved critical care management and treatment with anti-seizure medication (DeLorenzo et al., 2009; Lawrence and Inder, 2010). Using the 10-20 EEG channels monitoring which began before the use of anti-seizure medication, it has been highlighted that neonatal status epilepticus is primarily generated by severe acute brain injuries such as hypoxic-ischaemic encephalopathy and periventricular leucomalacia and that neonatal status epilepticus seems to be a predictive risk factor for epilepsy in preterm neonates ≤ 29 weeks gestation and in term neonates (Pisani et al., 2007).

Status epilepticus can also occur in neonatal stroke (Rafay et al., 2009); the outcome from these neonates has yet to be determined. Death due to status epilepticus is usually attributable to the underlying cause and not due to the prolonged seizure activity *per se* (Thibeault-Eybalin et al., 2009). However, while seizure duration and age onset of seizures have been implicated in the prognostic value of determining mortality due to status epilepticus, not all aetiologies play a crucial role in determining outcome (Pisani et al., 2007).

Based on seizure burden quantified using the continuous video-electroencephalography monitoring in 259 infants and children (51% male) with a median age of 2.2 years (interquartile range: 0.3 days-9.7 years), seizure burden lasting longer than 12 minutes in a given hour was strongly associated with neurological decline, thus supporting the hypothesis that electrographic seizures independently contribute to brain injury and worsen outcome regardless of what the aetiologies were (Payne et al., 2014).

Status epilepticus represents the most severe expression for seizures and has the potential to cause brain injury in the immature brain (Lawrence and Inder, 2010). In older children and adults, apart from the 30-minute rule, the presence of 2 consecutive seizures, or repeated seizures with brief intervals and most imperatively during which the child is unable to regain full consciousness is also included in the definition for status epilepticus (Panayiotopoulos, 2004). However, the application of this definition in neonates is obscure as the level of consciousness can be difficult to gauge, particularly if sedative medication is given (Volpe JJ, 2008).

It can be difficult to determine mental status in neonates; therefore choosing this criterion for definition as a clinical end-point to define status epilepticus in neonates is inappropriate. Furthermore by nature, neonatal EEG seizures are usually recurrent brief events rather than long and uninterrupted events (Clancy, 2006a). Hence, the criteria used to define status epilepticus which includes the level of consciousness, such as those applied in the more mature central nervous system in older children and adults are not particularly useful for neonates (Lawrence and Inder, 2010).

4.4 Neonatal seizure burden

Seizure burden is defined as the total duration of recorded electrographic seizures in minutes (Clancy and Legido, 1987). Seizure burden is an important factor to consider for outcome studies because prolonged or recurrent seizures are thought to be associated with poor long-term neurodevelopmental outcome (Clancy, 2006a; Payne et al., 2014; Pisani et al., 2007; Pisani et al., 2008). Increasing seizure duration, such as continuous or repetitive seizures, carries a higher risk for seizure-induced brain injury (Holmes and Ben-Ari, 2001), therefore EEG documentation to aid in the diagnosis is essential. The results of devastating physiological, metabolic, excitotoxic and genetic effects of electrographic seizures are dependent on the severity of each insult, as it has been shown that perinatally asphyxiated neonates with abundant seizures demonstrated a worse outcome (Maartens et al., 2012; Nunes et al., 2008; van der Heide et al., 2012).

Seizure burden is often relied upon to assess the response of neonates to anti-seizure medication or to determine the effectiveness of anti-seizure medication. Aggressive use of anti-seizure medication without EEG confirmation contributes to the inaccuracy in estimating the severity of seizures in neonates and medication-induced brain injury; furthermore intractable seizures require the use of multiple anti-seizure medication to control seizures (Boylan et al., 2004; Painter et al., 1999). Medication may also impede the recognition of persistent seizures due to the phenomenon known as “electroclinical dissociation of seizures” (previously discussed in Chapter 3).

The comparative effectiveness of different anti-seizure medication at varying gestational ages has not yet been fully established using EEG criteria. Seizure durations can be used as quantitative measures of anti-seizure medication effectiveness and seizure severity. Interestingly, the Neurology Group on Neonatal Seizures had proposed to define the effectiveness for seizure-reducing medication

as a total cessation of seizures of not merely to a 50% reduction of seizures (Clancy, 2006b), but beyond 50% in the reduction of seizures. Some authors have shown that seizure activity stopped within 6 hours of giving anti-seizure medication in neonates, with serum values within the accepted therapeutic range in all neonates (Malik et al., 2003; NEMO study, 2010; Slaughter et al., 2009).

In the last 30 years, various reports on seizure burden in neonates have been described. Up until a decade ago, the burden of neonatal seizures in critically ill newborns remained high; few studies have computed the minimal or maximal seizure durations in neonates (Bye and Flanagan, 1995b; Clancy et al., 1988; Scher et al., 1993). Clancy *et al.* quantified seizure duration in neonates with a variety of acute encephalopathies from EEG recording (length of recording ranged from 27 minutes to 3 hours) (Clancy and Legido, 1987). Seizures were best characterized as recurrent, but relatively brief events with a mean duration of only 137 seconds. The majority of seizures were no more than 9 minutes long.

Standard EEG monitoring which consists only of an hour recording undertaken at most clinical practices could potentially miss some of the EEG seizures. Clancy *et al.* used several quantitative measures of seizure burden *i.e.* mean (range) number of seizures per hour was 9.5 (1 to 66), mean seizure duration was 132 (19 to 675) seconds, mean longest recorded seizure was 280 (26 to 1840) seconds and the mean percentage of each EEG record during any channel which showed electrographic seizure activity was 23 (2 to 87)% (Clancy and Legido, 1991).

4.5 Conclusion

The characteristics of neonatal seizures differ from those of older children and adults. The information on seizure burden in neonates has clinical significance in terms of treatment and prognosis; however it needs to be revisited and re-explored as the definition of neonatal seizures can no longer be based only on clinical grounds which are notoriously known to be subjective. Current information on seizure burden in neonates has to be based on seizures detected by the gold standard: by prolonged continuous multichannel video-EEG recording. This forms the aims of my thesis which is to describe in more detail the matrixes in seizure burden as represented by our current population of neonates in our NICUs (described in Chapter 1).

Section 2

Methodology

Chapter 5

Methodology



Introduction

This research study was a prospective cohort study of neonates who were monitored with early, prolonged and continuous multichannel video-EEG because they were at risk of seizures in the first few days after birth. In this chapter, the study setting, recruitment process, EEG recording methods, study definitions, data collection, storage and analysis are discussed.

5.1 Study setting

5.1.1 Study population

Study site: Neonates were recruited from the neonatal units at Cork University Maternity Hospital (CUMH), Ireland and at Elizabeth Garrett Anderson Wing, University College London Hospital (UCLH), London, United Kingdom.

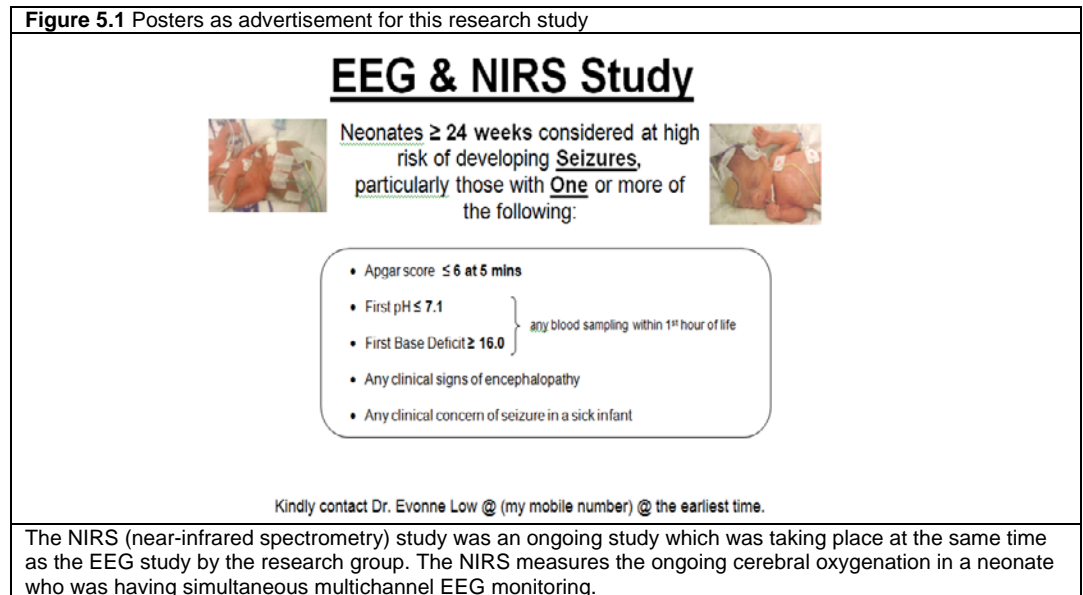
Study period: between 5th January 2009 until 31st December 2011 (3 years).

Inclusion criteria for neonates: Neonates ≥ 37 weeks gestation were enrolled for multichannel EEG monitoring if they fulfilled 1 or more of the following criteria:

1. Apgar score less than 6 at 5 minutes.
2. First ph of ≤ 7.1 (cord, capillary, venous or arterial blood sample).
3. Clinical evidence of encephalopathy, or
4. Any clinical concern of seizures on a sick neonate

Exclusion criteria: No parental consent.

Posters (figure 5.1) as advertisement for this research study were placed in the neonatal intensive care unit, special care baby unit, the Tutorial Room, the Doctors' On-Call rooms, in addition to word-by-mouth to doctors and nurses during my off and on-call clinical work as well as during EEG rotating hours.



5.1.2 Standard protocol approvals, registrations and patient consents

This study was conducted with approval from the Clinical Research Ethics Committees of the Cork Teaching hospitals, Ireland and the National Health Service in the UK, via the Integrated Research Application Service. Written, informed consent was obtained from at least one parent of each neonate who participated in this study.

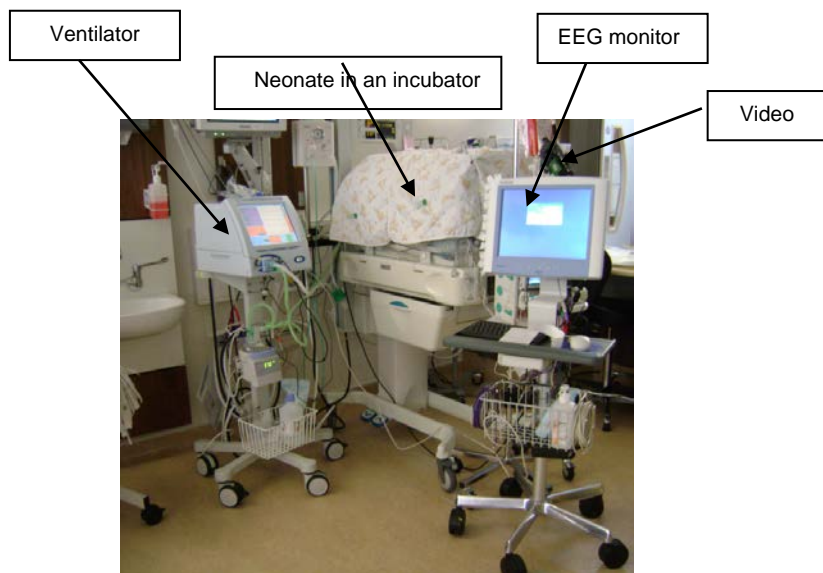
5.2 Electroencephalogram (EEG) recording in the neonatal unit

When neonates fulfilled the inclusion criteria for the study and written, informed consent was obtained from one of the parents, they were then monitored with multichannel video-EEG as soon as possible after birth. A bedside 12 channel Nicolet video-EEG monitor (CareFusion NeuroCare, Wisconsin, USA) was used to record the multichannel video-EEG (figures 5.2 and 5.3).

Figure 5.2 Nicolet multichannel video-EEG monitoring device



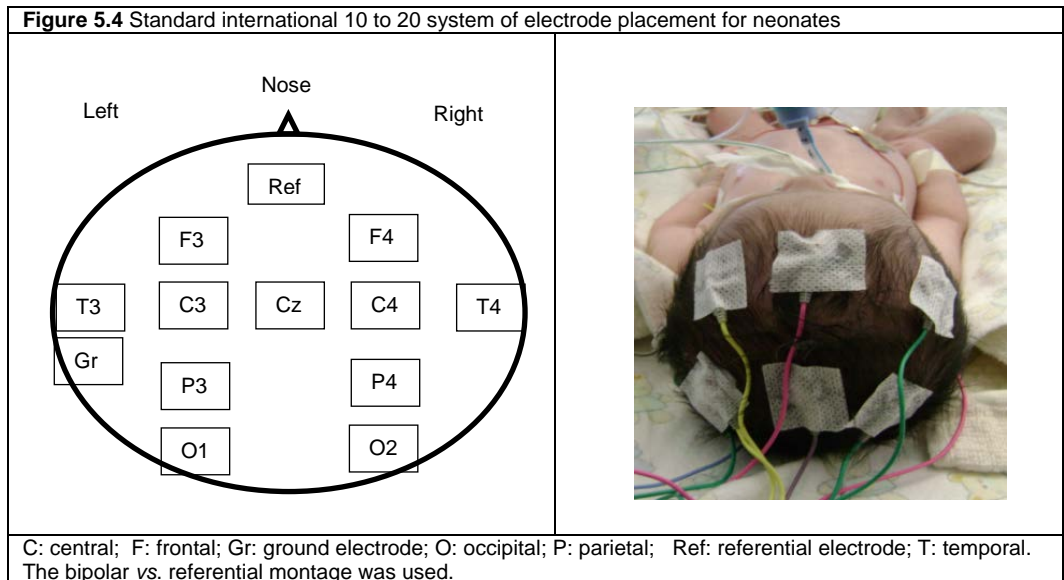
Figure 5.3 Multichannel video-EEG setting in the neonatal intensive care unit



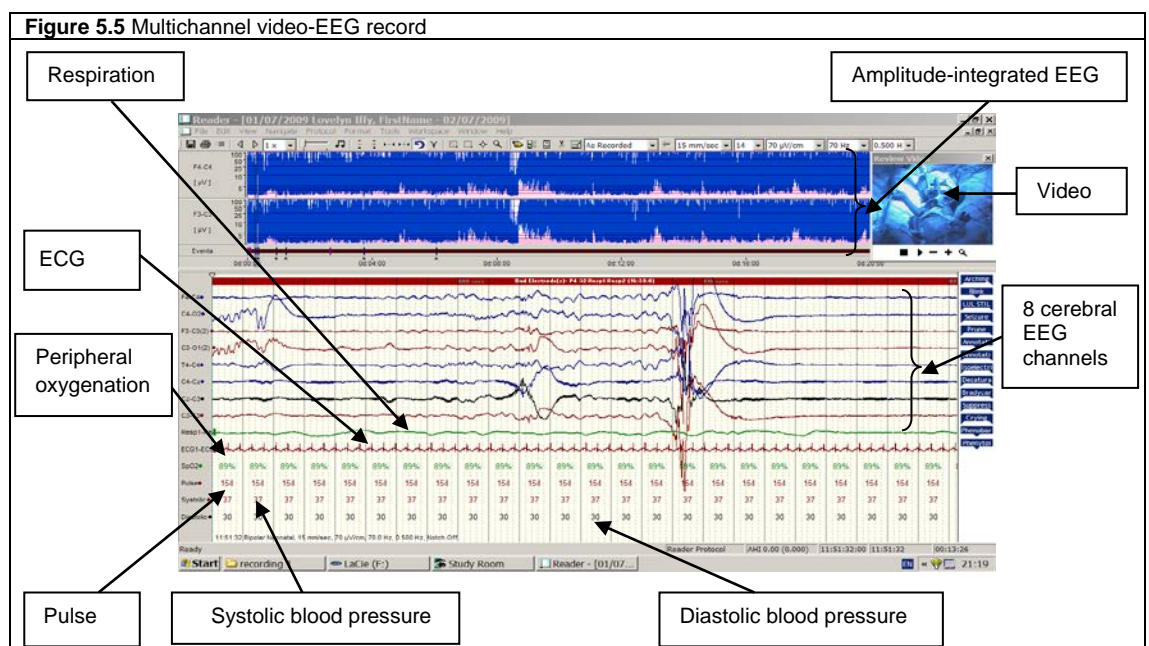
5.2.1 Scalp electrode placements

The preparation for this procedure involves a number of steps. The skin on the neonatal scalp was cleaned by removing any debris and skin cells using an alcohol wipe (Alcowipe). An abrasive gel acting as a skin prepping gel (NuPrep) and a soft EEG conductive paste (Ten20) were applied onto the scalp using the tip of a cotton bud, to achieve an impedance of less than 10 kOhms. Reducing the impedance with a gentle abrasive gel to less than 10 kOhms greatly enhances the quality of the EEG recording. A delicate tape (Mefix) was used to adhere the EEG electrodes (made of silver/silver chloride) onto the skin where the allocated site was cleaned on the neonatal scalp and a CPAP (continuous positive airway pressure) cap was used to keep the electrodes in placed.

Using the standard international 10 to 20 system of electrode placement modified for neonates (Klem et al., 1999), scalp electrodes were placed at F3, F4, C3, C4, T3, T4, O1, O2 and CZ, to record electrical activity from the frontal, central, temporal and occipital areas (figure 5.4). This reduced channel system has been previously shown to accurately record background EEG activity and neonatal seizure discharges (Tekgul et al., 2005).



Continuous digital video imaging of the neonate was recorded simultaneously. Physiological parameters such as heart rate, respiration, peripheral oxygen saturation and invasive arterial blood pressure (where available) were recorded digitally from the IntelliVue MP70 Neonatal monitor (Philips, Boeblingen, Germany). These physiological vital signs were recorded and stored simultaneously with the EEG signal on the Nicolet video-EEG monitor (figure 5.5).



In order to obtain good quality continuous EEG recordings for prolonged periods, regular checks of electrode placements were required to ensure that they were still intact and that the impedance of electrodes was maintained. Scalp electrodes, impedance and EEG traces were examined frequently during the recording and replaced and adjusted as necessary. If the neonate needed to be relocated within the neonatal unit, for example from the resuscitaire to an incubator, or if the neonate had procedures performed which potentially might disrupt the maintenance of the EEG electrodes (such as cranial ultrasound, x-ray), my colleagues and myself were informed by the nursing or medical personnel to assist in moving the EEG equipment and re-attach the EEG electrodes so as to avoid any loss of data. If the neonate was clinically unstable or needed to be transferred to another hospital for further treatment, we were be summoned to abort the EEG monitoring earlier than anticipated.

5.2.2 Visual Analysis of EEG

EEG analysis by visual inspection: The entire multichannel EEG recording from each neonate was independently reviewed by two experienced neurophysiologists, students analyzing the EEG and myself.

5.3 Radiographic features

Magnetic resonance imaging (MRI) studies were performed in a Siemens Avanto 1.5 Tesla unit (Siemens Ag, Erlangen, Germany) and computed tomographic (CT) scanning was performed using a Toshiba Aquilion 4-detector row CT (Toshiba, Tochigi-ken, Japan). All imaging studies were performed without sedation. Neonates were transferred to the MRI scanner in an MRI-compatible incubator with integrated neonatal array coils (MR Diagnostics Incubator, Lammers Medical Technology GmbH, Luebeck, Germany). The arterial territory and estimated size of cerebral infarction based on methods described by Marks *et al.*, (Marks et al., 1999) were reported by an experienced paediatric radiologist.

5.4 Standard protocol for treatment

The clinical management of neonates in terms of treatment including therapeutic hypothermia by whole body cooling, anti-seizure medication and other medication and was at the discretion of the attending neonatologist and was not be dictated by the clinical research team. All clinical seizures were treated as well as seizures recognized by the clinical team interpreting the aEEG. The aEEG used to confirm suspected seizures was also used as an aid in clinical decision-making at the cotside. Concerns regarding any abnormal behaviour or aEEG pattern prompted a

review of the multichannel EEG from the neurophysiologist in each hospital. Immediate reporting of the multichannel EEG was not always available; the aEEG and clinical suspicion were the mainstays of seizure confirmation.





5.4.1 Therapeutic hypothermia by whole body cooling

Neonates were cooled if they met the entry criteria for the UK Total Body Hypothermia for Neonatal Encephalopathy (TOBY) cooling registry (Azzopardi D et al., 2007) (figure 5.6). Outborn neonates received passive cooling prior to transfer with the overhead radiant warmer turned off. During transport the neonate will be nursed in a transport incubator. The incubator heater will be turned on and adjusted if necessary to maintain the rectal temperature between 33 and 34°C.

Figure 5.6 Cooling a neonate with multichannel video-EEG monitoring in the neonatal intensive care unit. Permission to be photographed obtained from neonatal nurse Maura Cahill



Figure 5.7 Equipment used for therapeutic hypothermia

| | |
|---|--|
| <p>a) Tecotherm TS med 200 machine (Tec-Com, Halle, Germany)</p>  | <p>b) CritiCool MTRE machine (Charter Kontron, Milton Keynes, UK)</p>  |
| <p>c) A cooling mattress is used to wrap the neonate when using the Tecotherm</p>  | <p>d) A CureWrap garment is used to wrap the baby when using the CritiCool machine</p>  |

Cooled gel packs will be placed around the neonate if necessary to maintain the target temperature. For active cooling, either the Tecotherm TS med 200 machine (Tec-Com, Halle, Germany) or the CritiCool MTRE machine (Charter Kontron, Milton Keynes, UK) was used (figure 5.7). Neonates were actively cooled to a rectal temperature of 33 to 34°C for 72 hours (unless contraindicated) and were slowly rewarmed by increasing the core temperature not more than 0.5°C per hour until it reaches the normal body temperature.

5.4.2 Anti-seizure medication

Phenobarbitone was the first-line anti-seizure medication administered to a maximum dose of 40 mg/kg intravenously. Second-line anti-seizure medication was administered if clinical and/or electrographic seizures recurred following phenobarbitone administration. In both hospitals, second-line anti-seizure medication was either intravenous phenytoin or midazolam.

Although standardized protocols for the use of anti-seizure medication were similar in both hospitals, the choice of second-line anti-seizure medication administration was at the discretion of the attending neonatologist. The timing and dose of each anti-seizure medication as well as morphine administered were recorded in all neonates.

5.5 Dataset for each study

The pathway in which neonates were included in each study is shown in a flow diagram at the Result section of each study.

Cooling study: The concluding diagnosis of hypoxic-ischaemic encephalopathy was at the discretion of the attending neonatologist. Every neonate was assigned a clinical grade of encephalopathy using the modified Sarnat score at 24 hours of age (Evans DJ et al., 1999). Non-cooled neonates were enrolled between June 2003 to September 2006 and January 2009 to March 2010 from the neonatal intensive care unit at Cork University Maternity Hospital (CUMH), Ireland. Cooled neonates were enrolled between January 2009 to September 2010 from CUMH and University College London Hospital (UCLH), United Kingdom.

Stroke study: The diagnosis of perinatal arterial ischaemic stroke was based on neuroimaging evidence of focal infarction affecting at most two arterial territories. Study analysis included only neonates with perinatal arterial ischaemic stroke who had electrographic seizures. Neonates with hypoxic-ischaemic encephalopathy,

infections, inborn errors of metabolism, blood disorders, venous or multiple infarctions were excluded due to differing pathogeneses and clinical manifestations when compared to those with focal arterial infarction.

Phenobarbitone and Electroclinical dissociation studies: The exclusion criteria for these studies were any neonates with a total EEG record of less than 12 hours in length, or with more than 50% of artefacts on the total EEG record, or with more than 36 hours gap between first 2 consecutive EEG records, or if the first EEG record was less than 12 hours, or any neonates with less than 8 cerebral EEG channels for more than 50% of the total EEG record or any ex-preterm neonates with EEG record done at term equivalent age. Further exclusion criteria specifically relating to EEG recordings were the exclusion of the last EEG when the previous EEG was done more than 36 hours ago, or when the EEG record had more than 50% of artefacts, or when an EEG record with less than 8 cerebral EEG channels for more than 50% of the total EEG record.

Randomization process for Phenobarbitone and Electroclinical dissociation studies
Assuming a kappa coefficient of 0.85, 70 neonates were required to be assessed by the expert rater in order to achieve a 95% confidence interval for kappa with a width of 0.2. Therefore for statistical validation reasons, the study sample size of 70 term neonates was deemed adequate. Between the 5th January 2009 and 30th June 2011, there were 192 term neonates (163 CUMH, 59 UCLH) who were consecutively recruited for EEG monitoring, 95 neonates were excluded. Fifty-six neonates were excluded because they had less than 12 hours EEG recording; 10 neonates had more than 50% of artefacts on the EEG and 29 neonates (17 had electrographic seizures) were excluded for further testing of the algorithm. Of the remaining 97 term neonates, 62 were “non-seizure neonates” and 35 were “neonates with seizures”. Thirty-five neonates were randomly selected from the 62 “non-seizure neonates”.

A total of 70 term neonates (35 non-seizure neonates and 35 neonates with seizures) were identified by the human expert rater. Each of these 70 neonates was given a coding system randomly. Neonates with multichannel EEG monitoring selected for the study analysis were those who were at high risk of having seizures or were treated with anti-seizure medication; these included some neonates who were without seizures but were treated with anti-seizure medication. Neonates who had at least one dose of anti-seizure medication administered during ongoing electrographic seizures were included for the Phenobarbitone study. Neonates

were excluded if all their anti-seizure medication doses were administered without evidence of electrographic seizures.

5.6 Clinical data collection

Clinical information

Clinical information of each neonate recruited for EEG was entered on Microsoft Excel 2007-2010 version. Every neonate who fulfilled the criteria for EEG monitoring was assigned a code systematically as they were enrolled consecutively, either in CUMH (prefixed by C_number) or UCLH (prefixed by L_number). The individual names of the neonates were only documented and stored in a concealed place at the Neonatal Research Centre in CUMH. The information details on the Excel can only be traced by a coding system.

EEG recordings

All EEG recordings with their video recordings were initially backed up and stored onto an external universal serial bus (USB). An electronic storage system for EEG records was set up by the Centre for Unified Computing, Boole Centre for Research in Informatics in UCC. All EEG records of every neonate were uploaded onto a UCC server available at the network location (1) [wellcome\(\\cucfs1.ucc.ie\)\(z:drive\)](http://wellcome(\\cucfs1.ucc.ie)(z:drive)), according to the assigned coding system. Data were updated on a regular basis.

Seizure burden annotations from human raters

All seizure annotations were initially made on the raw EEG record and were exported to a Notepad format (.txt as this is the only method Nicolet is able to generate all annotations currently). The .txt files were then transferred and opened with Excel format for more convenient calculations to be made. Further robust statistical analysis can be made by transferring the data from Excel onto a statistical package format. All data were stored and backed up securely in an external USB.

5.7 Definitions

An electrographic seizure was defined as a sudden and evolving repetitive stereotyped waveform with a definite start, middle and end, lasting for at least 10 seconds and with a minimum amplitude of two microvolts (Clancy and Legido, 1987) on at least one EEG channel.

Status epilepticus was defined as continuous or accumulative electrographic seizure activity lasting greater than 50% of a one hour period (Ortibus et al., 1996).

The electrographic seizure window was defined as the timepoint between the first and last recorded electrographic seizure in hours.

The recorded seizure burden was defined as the total duration of recorded electrographic seizures in minutes. It was also expressed in terms of seizure per hour and was calculated using a formula:

$$\text{Seizure burden} = \frac{\text{total seizure burden (minutes)}}{\text{electrographic seizure window (hours)}}$$

Seizure number was counted as the number of seizure events recorded on the EEG.

To avoid neonates with many seizures having much influence on the results, summary measures were calculated for each neonate. These summary measures were percentages of the number of seizure events and the seizure burden (seizure duration in minutes) associated with electroclinical seizures, electrographic-only and the duration when viewing of the video was obscured (for example during a medical procedure); they were calculated relative to the total number of electrographic seizures and the total seizure burden (seizure duration in minutes). For example:

$$\% \text{ number of electroclinical seizures} = \left(\frac{\text{the number of electroclinical seizures}}{\text{the total number of seizures}} \right) * 100$$

$$\% \text{ seizure burden of electroclinical seizures} = \left(\frac{\text{the seizure burden of electroclinical seizures}}{\text{the total seizure burden}} \right) * 100$$

% number of electrographic-only seizures= (the number of electrographic-only seizures/ the total number of seizures) * 100

% seizure burden of electrographic-only seizures= (the seizure burden of electrographic-only seizures/ the total seizure burden) * 100

Mean seizure duration was calculated for all recorded electrographic seizures in each neonate. In each neonate, the mean seizure duration is calculated as the proportion of the total seizure burden in seconds relative to the number of seizures:
Mean seizure duration= total seizure burden (in seconds) /total number of seizures

The entire **background EEG pattern** was graded and assessed for continuity, symmetry, synchrony, sleep cycling and other specific features.

Sleep cycling was assessed as being present, absent or disturbed in each neonate; a disturbed sleep cycling signified an interruption to the expected sleep cycle architecture of healthy term neonate (Lamblin et al., 2013).

Significant EEG suppression was defined as EEG activity below 5 µV in all EEG channels for at least 10 seconds respectively.

5.8 Statistical analysis

Detailed description of statistical analysis pertaining to the 4 different studies is found in the following chapters 6, 7, 8 and 9.

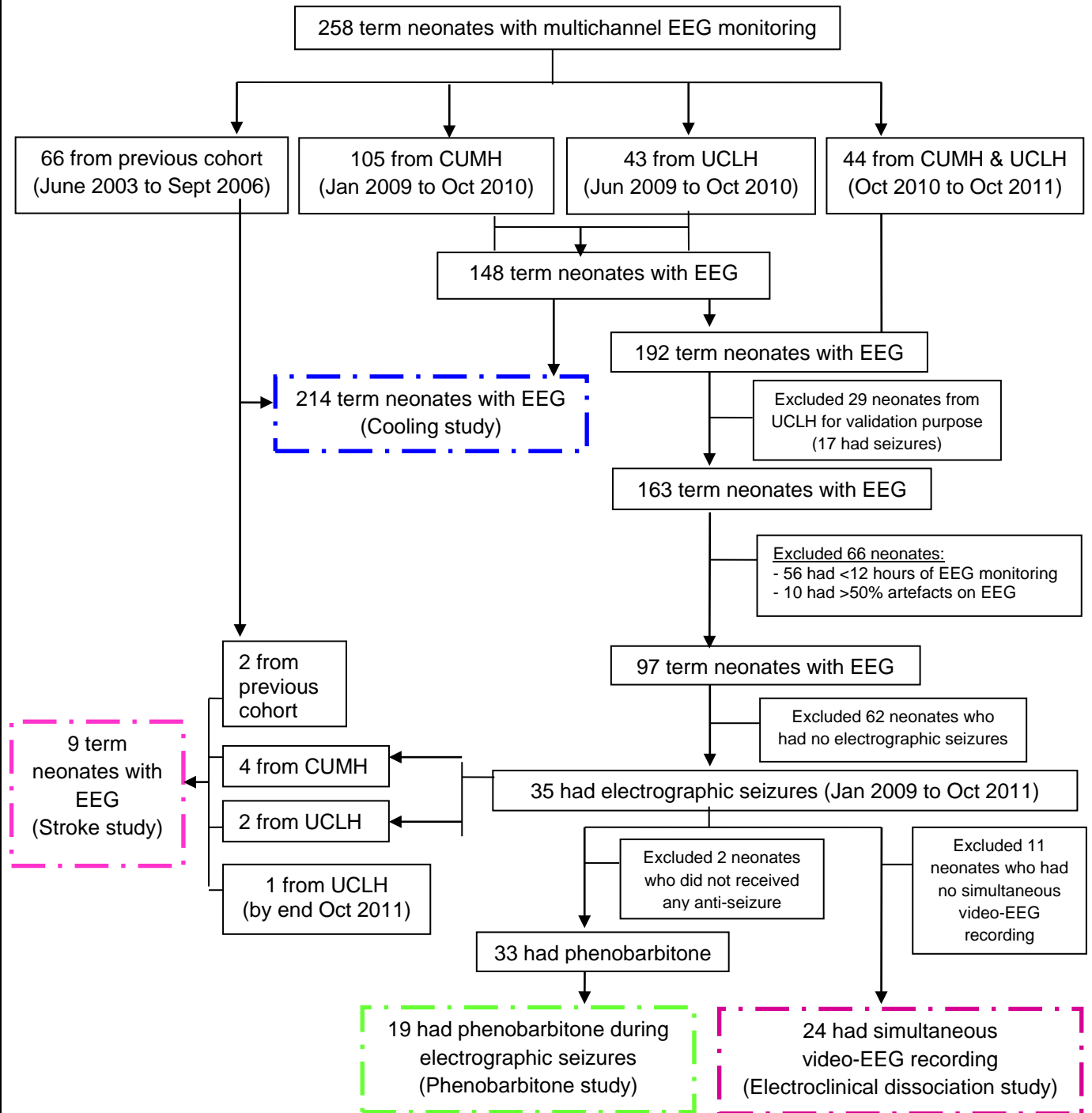
Section 3

Results and Discussions

Summary of dataset of neonates recruited for this research study

Diagram 3.0 Overall flow diagram on the recruitment timeline for this research study

Recruitment timeline: June 2003 to October 2011



CUMH: Cork University Maternity Hospital; UCLH: University College London Hospital, United Kingdom.

Chapter 6

Characteristics of Electrographic Seizure Burden in Term Neonates with Hypoxic-ischaemic Encephalopathy

6.1 Abstract

Background: Therapeutic hypothermia in neonates with hypoxic-ischaemic encephalopathy (HIE) improves long-term neurological outcomes.

Objective: To investigate the effect of therapeutic hypothermia on seizure burden, the recorded seizure burden was quantified based on multichannel video-EEG in HIE neonates who received therapeutic hypothermia and in those who did not.

Methods: Early, prolonged and continuous multichannel video-EEG recordings were performed and each EEG record was reviewed independently by two experienced electroencephalographers who were blinded to the allocation for therapeutic hypothermia. Comparison between the recorded seizure burden in non-cooled and cooled neonates was assessed. Data were expressed as medians and interquartile ranges (IQR).

Results: There were 107 neonates with HIE who were recruited for multichannel EEG monitoring during the study period of 2009 and 2011. Thirty seven neonates were identified to have electrographic seizures; of these, 31 had recordings that were suitable for analysis (16 non-cooled, 15 cooled). Compared with non-cooled neonates, earlier [age: 6 (3-9) vs 15 (5-20) hours] and longer [88 (75-101) vs 55 (41-60) hours] EEG monitoring were undertaken in cooled neonates. Despite this increased opportunity to capture seizures in cooled neonates, the recorded seizure burden in the cooled group was significantly lower than in the non-cooled group [60 (39-224) vs 203 (141-406) minutes; $p=0.027$]. Further exploratory analysis showed that the recorded seizure burden was only significantly reduced in cooled neonates with moderate HIE [49 (26-89) vs 162 (97-262) minutes; $p=0.020$] when compared with severe HIE.

Conclusions: Compared to neonates with severe HIE, a decreased seizure burden was noted in neonates with moderate HIE who received therapeutic hypothermia. This finding may explain some of the therapeutic benefits of hypothermia seen in term neonates with moderate HIE.

6.2 Introduction

Hypoxic-ischaemic encephalopathy (HIE) accounts for approximately 50 to 75% of neonatal seizures (Volpe JJ, 2008). Seizures have been shown to exacerbate pre-existing cerebral damage due to perinatal hypoxic-ischaemia (Yager et al., 2002). Outcome studies in neonates have shown that seizures are powerful predictors of morbidity and mortality (Glass et al., 2009; van Rooij et al., 2007). However, these studies relied almost entirely on the detection of seizures using clinical observation (Glass et al., 2009) or the amplitude-integrated electroencephalogram (aEEG) (van Rooij et al., 2007). It is well-known that many seizures can remain undetected by clinical recognition (Malone et al., 2009; Murray et al., 2008) or the aEEG (Glass et al., 2013; Rennie et al., 2004); therefore both methods cannot accurately quantify the precise seizure burden in neonates. Inherently, the accurate identification and quantification of neonatal seizures require early, prolonged and continuous monitoring with the multichannel video-EEG.

Based on many evidence of benefit from both animal and human studies, the National Institute for Health and Clinical Excellence has endorsed the use of therapeutic hypothermia for hypoxic perinatal brain injury in the United Kingdom (UK) (National Institute for Health and Clinical Excellence (NICE), 2010). Edwards *et al.* conducted a meta-analysis of three trials which had enrolled 767 neonates; they showed that therapeutic hypothermia reduced the combined rate of disability and death at 18 months (Edwards et al., 2010). Recently, longer term neurodevelopmental outcome of neonates who were treated with therapeutic hypothermia after perinatal asphyxia has shown that there was an improvement in neurocognitive function in children aged between 6 and 7 years old (Azzopardi et al., 2014).

However, the precise mechanism by which hypothermia achieves neuroprotection in neonates with HIE is unknown. In the biphasic model of neuronal death following hypoxia- ischaemia injury, the cascade of events which occurs in the secondary reperfusion injury phase may be associated with seizures, an accumulation of cytotoxins and failure of oxidative cerebral metabolism (Bennet et al., 2001; Busto et al., 1989; Tooley et al., 2003). Hypothermia may reduce the seizure burden in neonates by affecting or arresting some of these mechanisms during this vital phase of brain injury.

What is already known on this topic?

- Therapeutic hypothermia in neonates with hypoxic-ischaemic encephalopathy has been shown to ameliorate adverse long-term neurodevelopmental outcome at 18 months.
- Clinical recognition and the amplitude-integrated EEG can miss many seizures and therefore will underestimate the true seizure burden in neonates with hypoxic-ischaemic encephalopathy.
- Inherently, early, prolonged and continuous multichannel video-EEG monitoring provides the more accurate identification and quantification of electrographic seizure burden in term neonates.

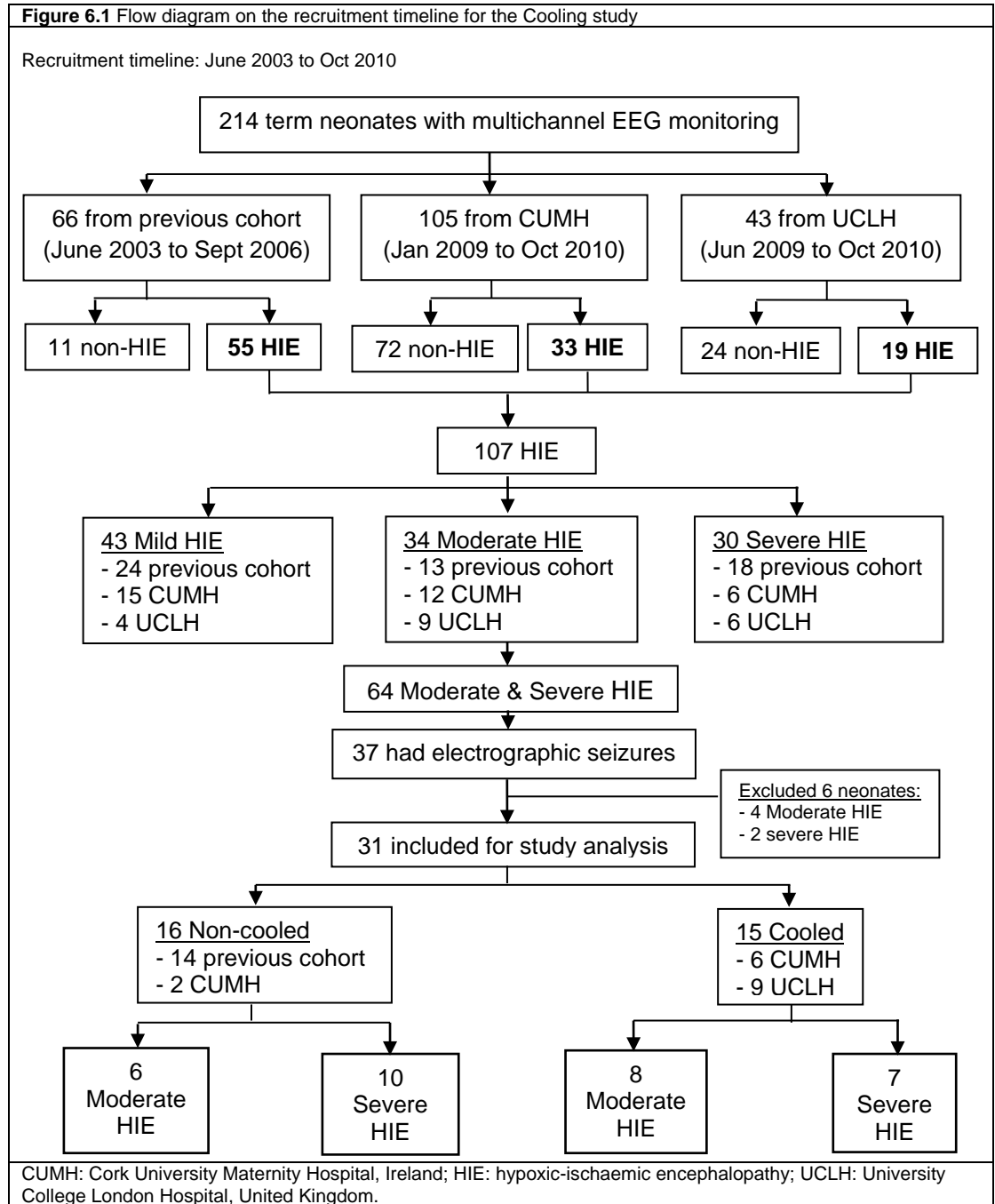
6.3 Aim

Hypothesis: Therapeutic hypothermia reduces seizure burden in term neonates with hypoxic-ischaemic encephalopathy (HIE).

Study aim: To determine the characteristics of seizures in neonates with hypoxic-ischaemic encephalopathy: non-cooled versus cooled neonates (the Cooling study). To date, therapeutic hypothermia has become the standard of care for neonates with hypoxic-ischaemic encephalopathy in most tertiary neonatal units, hence it is imperative to examine the seizure characteristics in cooled neonates with hypoxic-ischaemic encephalopathy. In this study, I investigated this by quantifying the effect of therapeutic hypothermia on recorded seizure burden obtained from continuous multichannel video-EEG monitoring.

6.4 Methods

Of the 214 neonates who had EEG monitoring, 66 neonates were from a historical cohort (55 of whom had HIE) and 148 neonates were from the current cohort (105 neonates from CUMH and 43 from UCLH). With the advent of therapeutic hypothermia, most of the historical cohort of neonates (2003-2006) were non-cooled and their EEG data were compared with neonates who were cooled in the current population (2009 to 2010). Neonates who had moderate and severe HIE were selected for this study analysis (figure 6.1). Further methods of EEG monitoring are described in Chapter 5 of Methodology section.



The recorded seizure burden was defined as the total duration of recorded electrographic seizures in minutes. Seizure number was counted as the number of seizure events recorded on the EEG. Mean seizure duration was calculated for all recorded electrographic seizures in each neonate.

Statistical analysis: The inter-rater agreement between 2 electroencephalographers was assessed using a Cohen's Kappa (κ) statistic. Continuous variables were described using medians and interquartile ranges (IQR) and categorical variables using frequencies. For comparisons between the two groups (non-cooled and cooled), the Mann-Whitney test was used for

continuous variables and the χ^2 test or Fisher's exact test (in the case of small expected counts) was used for categorical variables. All statistical analyses were performed using PASW Statistics 17.0. All tests were two-sided and a p value <0.05 was considered to be statistically significant.

6.5 Results

Patient population

During the study period between June 2003 to Oct 2010, there were 107 term neonates diagnosed with HIE (figure 6.1). Based on assessment using the clinical Sarnat grade for HIE, 43 neonates were identified to have mild HIE, 34 with moderate HIE and 30 neonates with severe HIE. From the 64 neonates with moderate or severe HIE, electrographic seizures were recorded in 37 neonates. Of these, 6 neonates were excluded from the study analysis: four neonates with moderate HIE were excluded [2 cooled neonates had secondary events shortly after EEG was commenced (one with cardiopulmonary arrest and the other with pulmonary haemorrhage), one cooled and one non-cooled neonate had less than 20 hours of artefact-free EEG] and 2 neonates with severe HIE were excluded (one cooled neonate with a subsequent principal diagnosis of mitochondrial respiratory chain disease and one non-cooled neonate with less than 20 hours of artefact-free EEG).

The remaining 31 neonates formed the study group (16 non-cooled, 15 cooled). Table 6.1 summarizes the clinical characteristics of neonates in both non-cooled and cooled groups. All non-cooled neonates were enrolled from CUMH (table 6.2). Nine of the 15 cooled neonates were enrolled from UCLH (table 6.3).

Table 6.1 Clinical characteristics of neonates included in this study

| | Non-cooled (n=16) | Cooled (n=15) | p value |
|--|----------------------|----------------------|---------|
| Gestational age (weeks) | 41 (40- 41) | 40 (40- 41) | 0.300 |
| Birthweight (grams) | 3 488 (3163- 3733) | 3275 (3000- 4130) | 0.707 |
| Gender (male: female) | 10: 6 | 9:6 | 0.886* |
| Clinical Sarnat score | | | |
| Moderate | 6 | 8 | 0.376* |
| Severe | 10 | 7 | |
| 5 minute Apgar score | 6 (2-8) | 4 (2- 4) | 0.050 |
| First pH | 7.134 (7.032- 7.217) | 6.930 (6.800- 7.100) | 0.009 |
| Number of anti-seizure medication | 2 (1-3) | 1 (1-2) | 0.274 |
| First-line anti-seizure medication (age in hours) | 12 (9-19) | 14 (10-24) | 0.504 |
| Total dose of first-line anti-seizure medication (mg/kg) | 30 (20-40) | 20 (20-20) | 0.203 |
| Second-line anti-seizure medication (age in hours) | 28 (24-31) | 26 (19-38) | 0.556 |
| Number of neonates on morphine | 8 | 15 | 0.002* |

Data are median (interquartile ranges) or n.

χ^2 test for the proportion of gender and clinical Sarnat score for neonatal hypoxia-ischaemic encephalopathy in non-cooled and cooled groups.

Table 6.2 Individual characteristics of non-cooled neonates with hypoxia-ischaemic encephalopathy

| Case | Clinical Sarnat score | Recorded seizure burden (minutes) | Seizure number (n) | Mean seizure duration (seconds) | Age at first EEG seizure | Age at first-line anti-seizure medication | Time from EEG seizure onset to treatment | Second-line anti-seizure medication | | Other drugs |
|---------------------|-----------------------|-----------------------------------|--------------------|---------------------------------|--------------------------|---|--|-------------------------------------|----------------|-------------|
| | | | | | | | | Age | Total dose | |
| C1 ^S | 2 | 38 | 4 | 574 | 10h 15m | None | N | | | M |
| C2 | 3 | 106 | 43 | 147 | 18h | | | 23h 14m | Pt= 10 mg/kg | M |
| C3 ^S | 2 | 116 | 21 | 331 | 26h 11m | 8h 5m | B | 25h 5m | Pt= 20 mg/kg | |
| C4 ^S | 3 | 137 | 84 | 98 | 14h 12m | None | N | | | M, Tr |
| C5 ^S | 2 | 152 | 99 | 92 | 22h 30m | 22h 34m | 4m | 32h 44m | Pt= 20 mg/kg | |
| C6 ^{E, S} | 2 | 172 | 21 | 493 | 25h 30m | 19h 13m | B | 28h 28m | Pt= 20 mg/kg | |
| C7 ^E | 3 | 183 | 121 | 91 | 12h 40m | 12h 20m | B | 35h 35m | Mz= 100 mcg/kg | M |
| C8 ^E | 3 | 199 | 41 | 291 | 17h 7m | 10h 50m | B | | | M |
| C9 | 3 | 206 | 60 | 206 | 12h 20m | 24h 24m | 12h 4m | | | M |
| C10 ^{E, S} | 2 | 212 | 66 | 193 | 10h 54m | 10h 10m | B | 28h 40m | Pt= 20 mg/kg | |
| C11 | 3 | 239 | 150 | 96 | 10h 56m | 19h 35m | 8h 39m | | | |
| C12 ^E | 3 | 384 | 209 | 110 | 21h 58m | 2h 30m | B | | Pt= 20 mg/kg | Cn, M |
| C13 ^{E, S} | 2 | 413 | 63 | 393 | 17h 54m | 10h 3m | B | | | |
| C14 ^{E, S} | 3 | 640 | 305 | 126 | 16h 48m | 18h 13m | 1h 25m | 29h 13m | Pt= 20 mg/kg | Pr, Mz, Py |
| C15 ^E | 3 | 958 | 190 | 303 | 27h 28m | 6h 34m | B | 9h 44m | Pt= 25 mg/kg | Mz, Py |
| C16 ^E | 3 | 1002 | 201 | 299 | 20h 35m | 16h 37m | B | 26h 47m | Pt= 40 mg/kg | M, D |

B: clinically treated before EEG commenced; C: neonates enrolled from the Cork University Maternity Hospital; Cn: clonazepam; D: intravenous diazepam; E: neonates with status epilepticus; M: morphine; Mz: midazolam; N: not given any anti-seizure medication; Pr: paraldehyde; Pt: phenytoin; Py: pyridoxine; S: neonates who were already seizing at the time when EEG was commenced; Tr: trichloral hydrate.

Table 6.3 Individual characteristics of cooled neonates with hypoxia-ischaemic encephalopathy

| Case | Clinical Sarnat | Recorded seizure burden (minutes) | Seizure number (n) | Mean seizure duration (seconds) | Age at first EEG seizure | Age at first-line anti-seizure medication | Time from EEG seizure onset to treatment | Second-line anti-seizure medication | | Other drugs | Cooling duration (age in hours) |
|-----------------------|-----------------|-----------------------------------|--------------------|---------------------------------|--------------------------|---|--|-------------------------------------|----------------|-------------|---------------------------------|
| | | | | | | | | Age | Total dose | | |
| L1 ^A | 2 | 19 | 4 | 283 | 10h 59m | 11h 30m | 31m | | | M | 72 (12-84) |
| L2 ^A | 2 | 24 | 17 | 85 | 11h 20m | 12h 25m | 1h 5m | 26h | Pt= 20 mg/kg | M, Mz | 72 (8-90) |
| L3 ^E | 2 | 31 | 2 | 917 | 6h 58m | 7h 25m | 27m | | | M | 72 (6-78) |
| C17 | 3 | 39 | 14 | 168 | 12h 22m | 6h 10m | B | | | M | 72 (0.25-72.25) |
| L4 | 2 | 48 | 12 | 241 | 24h 23m | 31h 44m | 7h 21m | | | M | 72 (6-78) |
| L5 ^{R, A} | 2 | 49 | 46 | 64 | 39h 26m | 24h 23m | B | | | M | 72 (9-81) |
| C18 ^E | 2 | 55 | 2 | 1658 | 8h 17m | 9h 55m | 1h 38m | | | M | 72 (2-74) |
| L6 ^W | 3 | 60 | 41 | 88 | 12h | 12h 51m | 51m | 20h 11m | Mz= 150 mcg/kg | M | 33 (0.5-33.5) |
| C19 | 2 | 100 | 22 | 274 | 13h 25m | 15h 38m | 2h 13m | | | M | 72 (2-74) |
| C20 | 2 | 118 | 76 | 93 | 21h 28m | 7h 20m | B | | | M | 72 (3-75) |
| C21 ^{E, W} | 3 | 214 | 56 | 229 | 16h 33m | 17h 3m | 30m | 28h 33m | Pt= 20 mg/kg | M | 65 (0.8-66) |
| L7 ^{R, W} | 3 | 224 | 281 | 48 | 12h 51m | 21h 22m | 8h 31m | 25h 52m | Mz= 340 mcg/kg | M | 23 (5-28) |
| C22 ^R | 3 | 244 | 185 | 79 | 42h 13m | 56h 25m | 14h 12m | 65h 35m | Pt= 20 mg/kg | M | 72 (6-78) |
| L8 ^{E, R, W} | 3 | 289 | 161 | 108 | 13h 5m | 27h 23m | 14h 18m | | | M | 19 (5-24) |
| L9 ^{S, W} | 3 | 421 | 178 | 142 | 10h 24m | 13h 35m | 3h 11m | 13h 55m | Mz= 330 mcg/kg | M | 66 (5-71) |

A: documented age onset of active cooling, passive cooling initiated earlier during transport; B: clinically treated before EEG commenced; C: neonates enrolled from Cork University Maternity Hospital; E: neonates with status epilepticus; L: neonates enrolled from University College London Hospital; M: morphine; Mz: midazolam; Pt: phenytoin; R: neonates with EEG seizures following discontinuation of cooling; S: neonates who were already seizing at the time when EEG record was commenced; W: shorter cooling period as part of withdrawal of life-sustaining support decision.

Therapeutic hypothermia and anti-seizure medication

Therapeutic hypothermia was commenced at the median (interquartile ranges) age of 5 (2-6) hours. In 6 of 7 cooled neonates with severe HIE, therapeutic hypothermia was commenced within 6 hours of age. However, due to clinical decisions to withdraw life-sustaining supportive care in these neonates, the duration of therapeutic hypothermia and EEG monitoring were shorter. A higher recorded seizure burden was noted in cooled neonates who had moderate HIE. Passive cooling was commenced earlier during transport to UCLH in 3 of 8 neonates with moderate HIE, but the recorded age at which active cooling commenced was beyond 6 hours of age. Despite this, all 8 neonates with moderate HIE received at least 72 hours of therapeutic hypothermia. Eight of 16 non-cooled neonates received at least one dose of phenobarbitone before EEG monitoring commenced.

All cooled neonates did not have any anti-seizure medication prior to EEG monitoring; all were only given anti-seizure medication during therapeutic hypothermia. However in both groups, there was no significant difference in the number of anti-seizure medication [non-cooled: 2 (1-3) vs cooled: 1 (1-2); $p=0.274$] and in the total administered dose of first-line anti-seizure medication [non-cooled: 30 (20-40) vs cooled: 20 (20-20) mg/kg; $p=0.203$]. There were no significant differences in the ages at which the first-line anti-seizure medication [non-cooled: 12 (9-19) vs cooled: 14 (10-24) hours; $p=0.504$] and the second-line anti-seizure medication administered [non-cooled: 28 (24-31) vs cooled: 26 (19-38) hours; $p=0.556$]. Based on the Fisher's exact test, all cooled neonates received morphine compared to 8 of 16 non-cooled neonates ($p=0.002$).

Characteristics of seizure burden

There was a high level of agreement in the interrater agreement for seizure identification ($\kappa=0.872$). In 8 non-cooled and 1 cooled neonates, seizures were noted to be ongoing when EEG recording commenced. The postnatal age of first recorded electrographic seizure was similar in both groups [non-cooled: 18 (12-22) vs cooled: 13 (11-22) hours; $p=0.252$]. In cooled neonates, the recorded seizure burden was significantly less than in the non-cooled group [60 (39-224) vs 203 (141-406) minutes; $p=0.027$] (table 6.4), while the number of seizures was fewer in the cooled than in the non-cooled group [41(12-161) vs 75 (42-180); $p=0.105$]. Cooled neonates had lower mean seizure duration than non-cooled neonates [142

(85-274) vs 200 (101-324) seconds; $p=0.192$], but these did not reach statistical significance.

Table 6.4 Characteristics of seizure burden in non-cooled and cooled groups

| All neonates | Non-cooled (n=16) | Cooled (n=15) | p value |
|--|-------------------|---------------|--------------|
| Recorded seizure burden (minutes) | 203 (141- 406) | 60 (39-224) | 0.027 |
| Total seizure numbers per neonate (n) | 75 (42-180) | 41 (12-161) | 0.105 |
| Mean seizure duration (seconds) per neonate | 200 (101-324) | 142 (85-274) | 0.192 |
| Number of neonates with status epilepticus (n) | 9 | 4 | 0.095* |
| Age onset of EEG (hours) | 15 (5-20) | 6 (3-9) | 0.006 |
| Total EEG duration (hours) | 55 (41-60) | 88 (75-101) | 0.001 |
| Moderate HIE | Non-cooled (n=6) | Cooled (n=8) | |
| Recorded seizure burden (minutes) | 162 (97- 262) | 49 (26-89) | 0.020 |
| Total seizure numbers per neonate (n) | 42 (17-74) | 15 (3-40) | 0.174 |
| Mean seizure duration (seconds) per neonate | 362 (168-513) | 258 (87-759) | 0.519 |
| Severe HIE | Non-cooled (n=10) | Cooled (n=7) | |
| Recorded seizure burden (minutes) | 223 (172-720) | 224 (60-289) | 0.558 |
| Total seizure numbers per neonate (n) | 136 (56-203) | 161 (41-185) | 0.591 |
| Mean seizure duration (seconds) per neonate | 137 (98-293) | 108 (79-168) | 0.172 |

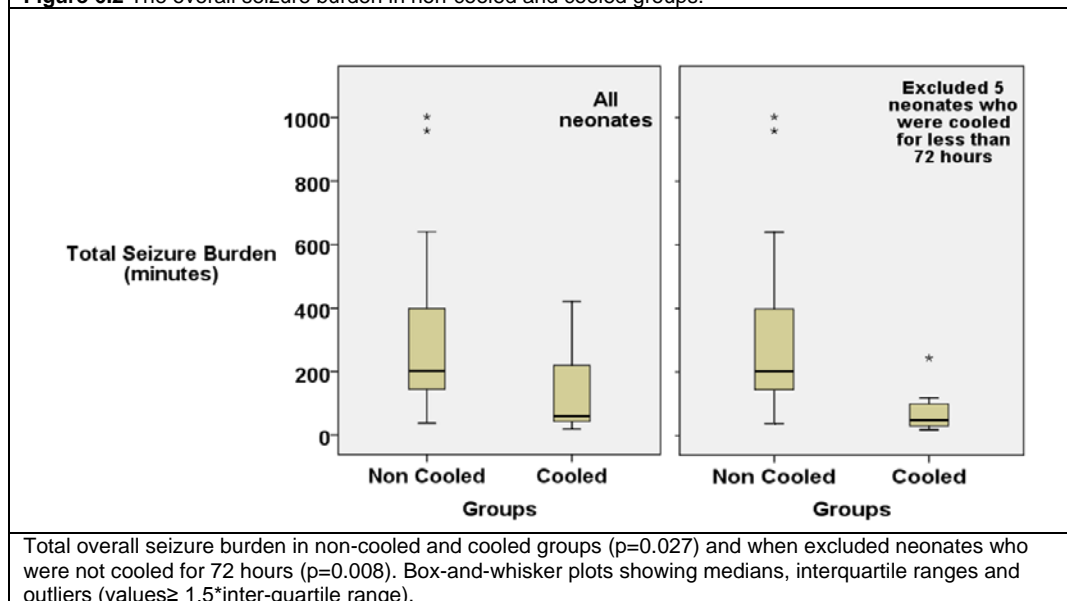
Data are median (interquartile ranges) or n.
 χ^2 test for the proportion of neonates with status epilepticus in non-cooled and cooled groups.

There were more neonates with status epilepticus in the non-cooled group when compared to the cooled group; this also was not statistically significant ($n=9/16$ vs $4/15$ cases, $p=0.095$). An exploratory subgroup analysis showed that therapeutic hypothermia had a significant reduction of recorded seizure burden in neonates with moderate HIE [non-cooled: 162 (97-262) vs cooled: 49 (26-89) minutes; $p=0.020$] while no such difference was seen in neonates with severe HIE [non-cooled: 223 (172-720) vs cooled: 224 (60-289) minutes; $p=0.558$].

Seizures following discontinuation of therapeutic hypothermia

Eleven cooled neonates had EEG monitoring long after therapeutic hypothermia was discontinued. When therapeutic hypothermia was discontinued, electrographic seizures were observed in 4 of 15 neonates (table 6.3). When a decision was made to withdraw life-sustaining supportive care, 2 of the 4 neonates had a shorter duration of therapeutic hypothermia (case L8- cases to tally with table for 19 hours, case L7 for 23 hours). In the remaining two cases (cases C22 and L5), electrographic seizures were observed following discontinuation of therapeutic hypothermia despite the fact that therapeutic hypothermia started at 6 and 9 hours respectively after birth and continued for 72 hours (figure 6.2).

Figure 6.2 The overall seizure burden in non-cooled and cooled groups.



6.6 Discussion

When compared to non-cooled neonates, this study has shown that there was a significantly lower electrographic seizure burden in term neonates with moderate and severe HIE when treated with whole body cooling. This is the first study to enumerate the seizure burden based on early and prolonged continuous multichannel video-EEG between non-cooled and cooled neonates.

Incidence of seizure burden since therapeutic hypothermia

In our study, we have found that the seizure burden was reduced in cooled when compared to the non-cooled neonates [60 (39-224) vs 203 (141-406) minutes]; this was significant in neonates with moderate HIE rather than those with severe HIE (Low et al., 2012a). Our findings were further confirmed by Srinivasakumar *et al.* who had added cranial magnetic resonance imaging findings to their study (Srinivasakumar et al., 2013). In this reserach study of neonates with moderate or severe HIE, the reported incidence of electrographic seizures in non-cooled and cooled cohorts were 54% and 61% respectively (and after study exclusion: 52% and 48% respectively) (Low et al., 2012a). These values are consistent with other studies using multichannel EEG (Nash et al., 2011; Rafay et al., 2009; Wusthoff et al., 2011).

However in the 2 recent hypothermia studies, (Nash et al., 2011; Wusthoff et al., 2011) the recorded seizure burden was not quantified and a control cohort (non-cooled) was not made available for comparison. In another study by Hamelin *et*

al., seizures had been shown to be less frequent in the cooled group but this was not significantly different with the non-cooled group (Hamelin et al., 2011). It would no longer be ethical to randomize neonates with HIE to normothermia.

Status epilepticus during therapeutic hypothermia

Not many studies have reported the occurrence of status epilepticus during cooling. Status epilepticus tends to occur in both neonates with moderate or severe HIE but more so in the non-cooled population (Low et al., 2012a). In a cooled cohort studied by Srinivasakumar *et al.*, in 5 (of 19) neonates with status epilepticus, all were noted to have severe brain injury as assessed by cranial magnetic resonance imaging. In a cohort of 56 neonates who were cooled, moderate to severe brain injury (as detected on magnetic resonance imaging at median age of 5 days) was more commonly detected in neonates with status epilepticus (5 of 17 neonates) (Glass et al., 2011b).

A study by Nash *et al.* also confirmed this finding in 4 of 15 cooled neonates who had status epilepticus and had moderate to severe brain injury (Nash et al., 2011). They concluded that during therapeutic hypothermia, seizures are a risk factor for brain injury particularly in neonates with status epilepticus. In the study by Wusthoff *et al.*, 23% of neonates continued to have status epilepticus (Wusthoff et al., 2011). Animal studies have advocated the use of therapeutic hypothermia as an adjunct to conventional anti-seizure medication to treat status epilepticus (Schmitt et al., 2006). Alternatively, a more effective anti-seizure medication acting as an adjunct to therapeutic hypothermia is much needed to control status epilepticus. Further reports relating to status epilepticus in the cooled population need to be assessed to determine whether there are any other factors involved apart from the severity of brain injury.

Electroclinical dissociation (ECD) of seizures during therapeutic hypothermia

Electroclinical dissociation (ECD) of seizures is common in neonates treated with therapeutic hypothermia (Nash et al., 2011; Wusthoff et al., 2011; Yap et al., 2009). Yap *et al.* monitored a cohort of 20 neonates (13 moderate HIE, 7 severe HIE) with selective head cooling (Yap et al., 2009). Concurrently with seizures on aEEG, initially 9 of 20 (45%) neonates were suspected of having clinical seizures at enrolment, during the first 24 hours of life during cooling, a total 18 of 20 (90%) neonates had non-convulsive seizures. Seizure burden increased in 9 of 20 (45%)

neonates during ongoing cooling period at 24 to 36 hours (day 2 to 3) of age. After 36 hours, seizure frequency decreased in 18 of 20 neonates. The occurrence of electrographic-only seizures in this study by Yap *et al.* is high. They had mainly used the aEEG and single-channel EEG tracing with intermittent multichannel EEG for seizure detection, and the use of anti-seizure medication with the occurrence of electroclinical dissociation of seizures was not discussed (All 20 neonates were given phenobarbitone and 14 of 20 neonates had received fosphenytoin and increasing midazolam infusion) (Yap *et al.*, 2009).

Using the gold standard: multichannel video-EEG from 26 neonates who received whole body cooling, (reviewed by 2 paediatric neurophysiologists), Wusthoff *et al.* detected 17 of 26 (65%) neonates who had electrographic seizures during and immediately after cooling; 8 of 17 (47%) had only electrographic-only seizures [13 of 17(76%) had seizure onset within 48 hours of life] and 4 of 17 (23%) had status epilepticus (Wusthoff *et al.*, 2011). In 10 of 26 (39%) neonates, phenobarbitone was given for suspected clinical seizures before EEG commenced [7 of 10 neonates received 1 dose of phenobarbitone; 8 of 10 neonates were treated based on electrographic seizures], 18 of 26 (69%) neonates were treated with anti-seizure medication (unspecified) during cooling. The treatment protocol in this study was to treat neonates with anti-seizure medication with the aim of total termination of electrographic seizures. The onset of seizures spanned from 6 to 95 hours of age; this study advocated that EEG monitoring should be extended beyond 24 hours for neonates receiving therapeutic hypothermia (Wusthoff *et al.*, 2011).

Using continuous video-EEG (commenced at 10.2 ± 2.9 hours of age for 90.9 ± 28.3 hours) and whole body cooling in 41 neonates treated with anti-seizure medication (lorazepam, phenobarbitone, fosphenytoin, levetiracetam), Nash *et al.* detected electrographic seizures in 14 of 41(34%) neonates [13 of 14 neonates had seizure onset within 18 hours of age, 8 neonates within 6 hours of age] and 4 of 41 neonates had status epilepticus (Nash *et al.*, 2011). Electroclinical seizures occurred in 8 of 14 (57%) neonates and electrographic-only seizures were noted in 6 of 14 (43%) neonates [3 of 6 neonates had status epilepticus]. However, the effect of anti-seizure medication on electrographic-only seizures was not assessed in studies by Wusthoff *et al.* (Wusthoff *et al.*, 2011) and Nash *et al.* (Nash *et al.*, 2011).

Perhaps differing treatment strategies in other institutions with the use of different first-line anti-seizure medication (lorazepam) may have explained the lower incidence of electrographic-only seizures in these studies. In a cohort of neonates who were cooled, the study by Glass *et al.* have shown that the electroclinical dissociation of the seizures is as common as electroclinical seizures (57% vs 60%) (Glass *et al.*, 2011b). These studies have shown that electroclinical dissociation of seizures is common in neonates with HIE receiving therapeutic hypothermia; these findings further reinforce the emphasis on the importance of using the multichannel EEG monitoring as part of routine clinical management.

Anti-seizure medication and therapeutic hypothermia

There may be a potential bias to the results in relation to the choice of anti-seizure medication used in this study. The timing and dose of anti-seizure medication may differ as the administration of medication was at the discretion of different attending neonatologists in both enrolling hospitals. Based on multicenter studies in Europe (Vento *et al.*, 2010) and in the United States of America (Bartha *et al.*, 2007), there is still no consensus on a standard protocol for the use of anti-seizure medication among neonatologists.

Compared to cooled group, the recorded seizure burden remained higher in the non-cooled group who had received more numbers and doses of anti-seizure medication. Despite this, the results from this research study have shown that there was no significant difference between the non-cooled and cooled groups with respect to the number, dose and age in hours when the first and second-line anti-seizure medication were administered. None of the cooled neonates and half of the non-cooled neonates had received at least one dose of phenobarbitone before EEG monitoring.

The synergistic anti-seizure medication properties of cooling with other anti-seizure medication have been described (previously discussed in Chapter 2). All but two non-cooled neonates in this research study received phenobarbitone, which remains the most commonly used first-line anti-seizure medication in most neonatal units (Bartha *et al.*, 2007; Vento *et al.*, 2010). The reduced effectiveness of this gamma-aminobutyric acid (GABA)-enhancing anti-seizure medication has been linked to the altered neuronal chloride transport in the developing brain (Dzhala *et al.*, 2005).

Sedative and anaesthetic medication have been shown to facilitate the therapeutic effects of hypothermia (Tooley et al., 2003). All cooled neonates and half of the non-cooled neonates received morphine (Low et al., 2012a). However, morphine itself does not possess anti-seizure properties; so this difference alone is unlikely to explain the measured difference in the recorded seizure burden between the non-cooled and cooled groups.

Effect of cooling on severe HIE

It is debatable whether severely affected HIE neonates would benefit from therapeutic hypothermia. The results of exploratory analysis in this research study showed that the recorded seizure burden was only significantly reduced in cooled neonates with moderate HIE. Possibly, this is related to the higher recorded seizure burden in 5 of the 7 cooled neonates with severe HIE who had shorter durations of therapeutic hypothermia and EEG monitoring following decisions to withdraw life-sustaining support.

Studies by Gluckman *et al.* (Gluckman et al., 2005) and Shankaran *et al.* (Shankaran et al., 2005) showed that cooling is most effective in neonates with moderate encephalopathy. Interestingly, the analysis of the 3 hypothermia trials in neonates has revealed that the primary outcome of neurodisability and death at 18 months was significantly reduced by cooling neonates with moderate HIE but not with severe HIE (Edwards et al., 2010). However, a recent study by Simbruner *et al.* has shown that therapeutic hypothermia was strongly neuroprotective for severe HIE (Simbruner et al., 2010).

In determining neonatal outcome from seizures or other brain insults, it is vital to know the pre-existing condition of the fetal or neonatal brain (Gluckman and Williams, 1992). The neurons in severe HIE which have undergone necrosis, may be rendered non-rescuable by therapeutic hypothermia. However, it is important to emphasize that further data are required to clarify whether therapeutic hypothermia is appropriate for severe HIE, before clinical decisions are made to abort cooling neonates with severe HIE. Efforts to supplement therapeutic hypothermia with other neuroprotective agents and to extend the neuroprotection window beyond 72 hours may prove useful for this vulnerable population of neonates (Aly et al., 2012; Charriaut-Marlangue et al., 2014; Dingley et al., 2014; Faulkner et al., 2011; Herrera et al., 2014; Robertson et al., 2013).

Rewarming seizures or seizures following discontinuation of therapeutic hypothermia

In this research study, eleven cooled neonates had EEG monitoring after therapeutic hypothermia was discontinued. Electrographic seizure events were observed in 4 of 15 cooled neonates when therapeutic hypothermia was discontinued. When a decision was made to withdraw life-sustaining support, two of the four cases had a shorter duration of therapeutic hypothermia. In the remaining two neonates, electrographic seizures were observed following discontinuation of therapeutic hypothermia despite the fact that therapeutic hypothermia started at 6 and 9 hours respectively after birth and continued for 72 hours.

Transient rebound epileptiform activity has been previously observed when hypothermia was discontinued after 72 hours (Gunn et al., 2005). Shah *et al.* has shown that in human term neonates, seizures are commonly noted during cooling (on day 1), however there seems to be a significant second peak of seizures rebounding during the rewarming period (on day 4) (Shah et al., 2014).

Duration of EEG monitoring

All non-cooled neonates would have qualified for cooling if therapeutic hypothermia was available at the time of recruitment. The time of onset and duration of EEG recording between non-cooled and cooled groups were significantly different. With the advent of therapeutic hypothermia, EEG monitoring has been the standard of monitoring and that cooled neonates were monitored longer when compared with the non-cooled cohort; thus creating a selection bias. Several non-cooled neonates were already experiencing seizures when EEG recording commenced; the recorded seizure burden in non-cooled group may have been underestimated. Despite this, and the fact that there was a longer EEG recording time which increased the possibility of capturing more seizures in the cooled group, the overall recorded seizure burden was still lower in the cooled group.

Two recent hypothermia studies have not quantified the recorded seizure burden and a control cohort was not made available for comparison (Nash et al., 2011; Wusthoff et al., 2011). cooled group but this was not significantly different with a control cohort (Hamelin et al., 2011). It would be ideal if the study was done

prospectively between cooled and non-cooled group, as this was conducted by Alistair Gunn's animal study (Gunn et al., 1997), however it would no longer be ethical to randomize human neonates with HIE to normothermia.

6.7 Conclusion

In neonates with HIE, this study has shown that therapeutic hypothermia was associated with a decreased recorded seizure burden. This effect may account for the reduction in neuronal damage due to seizures, and may help to explain the observed improvement in long-term neurological outcome seen in neonates with HIE who were treated with therapeutic hypothermia. Further studies using early, prolonged and continuous multichannel EEG monitoring for accurate determination of seizure burden are undoubtedly warranted in neonates receiving therapeutic hypothermia.

What this study adds?

- Using the multichannel EEG, this is the first study to report that the recorded electrographic seizure burden is decreased in neonates with hypoxic-ischaemic encephalopathy who were cooled, when compared with neonates who were not cooled.
- Therapeutic hypothermia may possess some anti-seizure properties, as it has the ability to reduce the electrographic seizure burden in term neonates who were cooled.

Chapter 7

Characteristics of Electrographic Seizures in Term Neonates with Stroke

7.1 Abstract

Purpose This chapter aims to describe specifically the characteristic EEG patterns in neonates with perinatal arterial ischaemic stroke (PAIS) and who had seizures.

Design Retrospective observational study of neonates >37 weeks gestation, born between 2003 and 2011 in two hospitals.

Methods Continuous multichannel video-EEG was used to analyze the background patterns and characteristics of seizures. Each EEG was assessed for continuity, symmetry, characteristic features and sleep cycling; morphology of electrographic seizures was also examined. Each seizure was categorized as electrographic-only or electroclinical.

Results Nine neonates with PAIS seizures and EEG monitoring were identified. While EEG continuity was present in all cases, the background pattern showed suppression over the infarcted side; this was quite marked (>50% amplitude reduction) when the lesion was large (>66% of one hemisphere). Characteristic unilateral bursts of theta activity with sharp or spike waves intermixed were seen in all cases. Sleep cycling was generally present but was more disturbed over the infarcted side. Seizures demonstrated a characteristic pattern; focal sharp waves/spike-polyspikes were seen at frequency of 1 to 2 Hz and phase reversal over the central region was common. There were more electrographic-only than electroclinical seizures (78 vs 22%).

Conclusions Cotside EEG monitoring in neonates with PAIS shows consistent electrographic features which could prove very useful for early diagnosis. Focal electrographic and electroclinical seizures with ipsilateral suppression of the background activity and focal sharp waves are strong indicators of PAIS. Approximately 80% of the total seizure burden resulted from clinically unsuspected seizures in neonates with PAIS. Prolonged and continuous multichannel video-EEG monitoring is advocated for adequate seizure surveillance.

7.2 Introduction

Stroke is the commonest identifiable aetiology of neonatal seizures after hypoxic-ischaemic encephalopathy (HIE) in the term neonate (Volpe JJ, 2008). Stroke can occur at any age but the incidence of stroke is particularly high during the 2 extremes of life; in the neonatal period and in the elderly adults. It is difficult to diagnose stroke in the newborn period as many neonates can be asymptomatic in the immediate neonatal period. However, it has been reported that approximately 60% of neonates who have had perinatal stroke, were symptomatic in the neonatal period; the remainder are identified after the neonatal period (*i.e.* beyond 28 days after birth) (Volpe JJ, 2008).

To date, the EEG is often used as the gold standard to detect seizures at the cotside. It is becoming a more useful tool for neonatologists because EEG abnormalities can also express antepartum, intrapartum and neonatal insults to the brain, thereby allowing proper assessment of the degree of severity in HIE. Although, EEG abnormalities are rarely pathognomonic for a specific clinical pathologic situation, our study aims to make every attempt to complement EEG studies with clinical history, examination and neuroimaging studies, to better define and distinguish the diagnoses between stroke and HIE in term neonates.

What is already known on this subject?

- Therapeutic hypothermia has been widely accepted as the standard of care for term neonates with hypoxic-ischaemic encephalopathy, but not for stroke.
- In the era of therapeutic hypothermia, seizure burden in neonates with stroke remains unknown.
- The characteristics electrographic seizures in neonates with stroke have never been described.

7.3 Aims

Hypothesis: There are characteristic features of electrographic seizures in neonates with stroke, hence potentially making the diagnosis earlier than other cranial imaging modality. In addition, in the absence of cooling, seizure burden in neonates with stroke may be higher than anticipated.

Study aim: Most studies have focused on seizures due to hypoxic-ischaemic encephalopathy and less is known about seizures caused by stroke, which remains the second most identifiable cause of seizures in term neonates. In this study, I describe the characteristic electrographic seizure burden and morphology of term neonates with stroke (the Stroke study).

7.4 Methods

Neonates for this study had presented with clinical seizures or suspected of having seizures; subsequently these seizures were confirmed on EEG monitoring. When a later neuroimaging (cranial ultrasound, CT or MRI) revealed that these neonates were confirmed to have stroke, their EEG recordings were selected for this study analysis. The methodology involved in this study has also been described in detail in Chapter 5 (Methodology).

The electrographic seizure window was defined as the timepoint between the first and last recorded electrographic seizure in hours.

The recorded seizure burden was defined as the total duration of recorded electrographic seizures in minutes. It was also expressed in terms of seizure per hour and was calculated using a formula:

$$\text{Seizure burden} = \frac{\text{total seizure burden (minutes)}}{\text{electrographic seizure window (hours)}}$$

Seizure number was counted as the number of seizure events recorded on the EEG.

To avoid neonates with many seizures having much influence on the results, summary measures were calculated for each neonate. These summary measures were percentages of the number of seizure events and the seizure burden (seizure duration in minutes) associated with electroclinical seizures, electrographic-only and the duration when viewing of the video was obscured (for example during a medical procedure); they were calculated relative to the total number of electrographic seizures and the total seizure burden (seizure duration in minutes). For example:

% number of electroclinical seizures= (the number of electroclinical seizures/ the total number of seizures) * 100

% seizure burden of electroclinical seizures= (the seizure burden of electroclinical seizures/ the total seizure burden) * 100

% number of electrographic-only seizures= (the number of electrographic-only seizures/ the total number of seizures) * 100

% seizure burden of electrographic-only seizures= (the seizure burden of electrographic-only seizures/ the total seizure burden) * 100

Mean seizure duration was calculated for all recorded electrographic seizures in each neonate. In each neonate, the mean seizure duration is calculated as the proportion of the total seizure burden in seconds relative to the number of seizures:
Mean seizure duration= total seizure burden (in seconds) /total number of seizures

The entire **background EEG pattern** was graded and assessed for continuity, symmetry, synchrony, sleep cycling and other specific features.

Sleep cycling was assessed as being present, absent or disturbed in each neonate; a disturbed sleep cycling signified an interruption to the expected sleep cycle architecture of healthy term neonate (Lamblin et al., 2013).

Significant EEG suppression was defined as EEG activity below 5 µV in all EEG channels for at least 10 seconds respectively.

Magnetic resonance imaging (MRI) studies were performed in a Siemens Avanto 1.5 Tesla unit (Siemens Ag, Erlangen, Germany) and computed tomographic (CT) scanning was performed using a Toshiba Aquilion 4-detector row CT (Toshiba, Tochigi-ken, Japan). All imaging studies were performed without sedation. Neonates were transferred to the MRI scanner in an MRI-compatible incubator with integrated neonatal array coils (MR Diagnostics Incubator, Lammers Medical Technology GmbH, Luebeck, Germany). The arterial territory and estimated size of cerebral infarction based on methods described by Marks *et al.*, (Marks et al., 1999) were reported by an experienced paediatric radiologist.

Statistical analysis: To avoid neonates with many seizures having much influence on the results, summary measures were calculated for each neonate (as discussed previously in section 5.4: Matrices of seizure burden). These summary measures were described across all neonates using medians and interquartile ranges (IQR). For paired comparisons, the Wilcoxon signed-rank test was used. All statistical analyses were performed using SPSS Statistics 20.0 (IBM SPSS Statistics, Illinois, USA). All tests were two-sided; p value <0.05 was considered to be statistically significant.

7.5 Results

During the study, nine neonates with PAIS who had early, prolonged and continuous multichannel video-EEG monitoring had electrographic seizures, and this is shown in the flow diagram in figure 7.1. Five neonates had coagulation testing and none had thrombophilic disorders. Table 7.1 lists the clinical demographics and outlines the MRI findings in eight of the nine neonates with various degrees of middle cerebral artery (MCA) infarction; one neonate had CT imaging. Cranial imaging was undertaken at median and interquartile ranges (IQR) of 5 (3-12) days after birth.

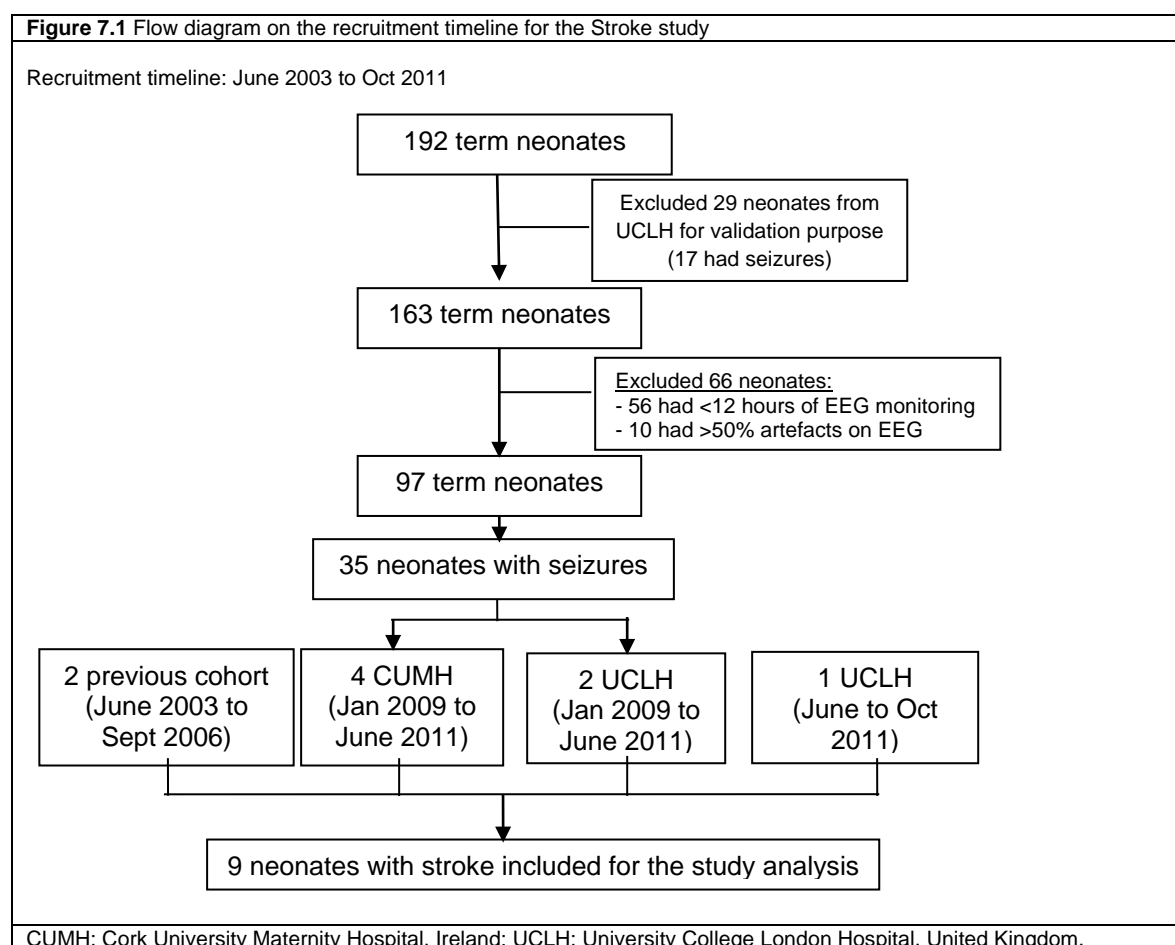


Table 7.1 Demographics and neuroimaging features of neonates in the order of increasing seizure burden

| Neonate | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|-------------|-------------------------------|--------------|---------------|-----------------------|----------------|--------------|---------------|----------------|
| Birthweight (grams) | 3700 | 3740 | 3750 | 3410 | 2830 | 3420 | 3160 | 3670 | 3480 |
| Gestation (weeks) | 40 | 39 | 41 | 41 | 39 | 41 | 39 | 41 | 41 |
| Gender | Male | Male | Male | Female | Female | Female | Male | Male | Male |
| Perinatal events | None | Polyhydramnios, PROM (36h) | NRCTG | None | NRCTG, PROM (>18h) | None | NRTCG | FTP | FTP |
| Mode of delivery | VV | VV | EMCS | Forceps | EMCS | Ventouse | EMCS | EMCS | EMCS |
| First pH | 7.42 | 7.04 | 7.13 | 7.29 | 7.00 | 7.34 | 7.30 | 7.41 | 7.27 |
| 5 min Apgar score | 10 | 9 | 9 | 10 | 6 | 7 | 10 | 10 | 10 |
| Age at first clinical seizure (hours) | 36 | 54 | 20 | 6 | 47 | 33 | 15 | 18 | 33 |
| First clinical seizure | RUL | Dusky episodes | LS | RS | LLL | RS | RUL | RUL | LUL |
| Age at EEG (hours) | 54 | 59 | 26 | 9 | 53 | 3 | 18 | 19 | 36 |
| Age at first recorded EEG seizure (hours) | 54 | 60 | 26 | 9 | 53 | 39 | 19 | 19 | 36 |
| EEG duration (hours) | 25 | 70 | 49 | 39 | 44 | 46 | 49 | 63 | 229 |
| Cerebral infarction | LMCA | LMCA | RMCA | LMCA, RMCA | RMCA | LMCA | LMCA | LMCA, LPCA | RMCA |
| Age at cranial imaging (days) | 5 | 8 | 29 | 3 | 10 | 3 | 2 | 3 | 14 |
| Estimated size of infarction (%) (mode of cranial imaging) | <33 (CT) | >66 (MRI) | <33 (MRI) | <33 (MRI) | 33-66 (MRI) | 33-66 (MRI) | <33 (MRI) | >66 (MRI) | 33-66 (MRI) |

CT: computerized tomography; EMCS: emergency Caesarean section; FTP: failure to progress; LLL: left lower limb clonic; LMCA: left middle cerebral artery; LPCA: left posterior cerebral artery; LS: left-sided clonic; LUL: left upper limb clonic movements; MRI: magnetic resonance imaging; NRCTG: non-reassuring cardiotocogram; PROM: premature rupture of membranes; RMCA: right middle cerebral artery; RS: right-sided clonic; RUL: right upper limb clonic; VV: vertex vaginal.

Table 7.2 summarizes the background EEG and seizure characteristics for each neonate. In all neonates, a continuous background pattern was present but voltage suppression and intermittent sharp theta discharges were seen over the infarcted side (figure 7.2). Background EEG suppression was greatest in cases where the estimated size of infarction was larger than 66% of one hemisphere. Sleep cycling was present in all cases but disrupted in many. The morphology of seizures in neonates with PAIS showed a characteristic pattern in all cases (figure 7.3). Spike and polyspike waves at a frequency of 1 to 2 Hz were seen over the infarcted side and phase reversal of these spikes over the central region was evident as the seizure evolved. Higher frequency temporal discharges were seen during apnoea in a neonate (case 2) who presented with dusky episodes. In this group of neonates with stroke, electrographic seizure spread to the contralateral side was not observed. On the non-infarcted side, no independent focal abnormalities were observed. Evidence of whether there may be any changes affecting short or long term outcome, if these electrographic seizures were eliminated was not analysed; but may be worth investigating in future research.

Table 7.2 Characteristics of EEG and seizures in neonates with perinatal arterial ischaemic stroke

| Neonate | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|--|--|---|---|--|--|--|---|--|
| Summary of background EEG features | | | | | | | | | |
| Continuous activity | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Symmetry | Left mild suppression | Left significant suppression | Right mild suppression | Good | Right mild suppression | Good | Good | Left significant suppression | Right mild suppression |
| Intermittent features | Left-sided sharp theta bursts | Left-sided theta sharp waves | Right focal sharp waves | Left-sided sharp waves in quiet sleep | Right-sided theta sharp waves | Left-sided theta sharp waves | Left-sided focal sharp theta waves | Left-sided theta sharp waves | Right-sided sharp waves |
| Sleep cycling | Normal bilaterally | Disturbed unilaterally | Disturbed bilaterally | Disturbed bilaterally | Normal bilaterally | Normal bilaterally | Disturbed unilaterally | Disturbed unilaterally | Normal bilaterally |
| Seizure morphology | Focal spikes over left central with phase reversal | Focal spikes over left central with phase reversal | Focal spikes over right central with phase reversal | Focal spikes & polyspikes over left central with phase reversal | Focal spikes & polyspikes over right central with phase reversal | Focal spikes over left central with phase reversal | Focal spikes over left central with phase reversal | Focal spikes & polyspikes over left central with phase reversal | Focal spikes & polyspikes over right central with phase reversal |
| Summary of seizure burden | | | | | | | | | |
| Total seizure burden (minutes) | 19 | 67 | 101 | 133 | 162 | 201 | 266 | 327 | 332 |
| Seizure burden (minutes/hour) | 2.70 | 7.28 | 27.60 | 5.53 | 10.27 | 18.15 | 12.77 | 9.25 | 6.18 |
| Mean seizure duration (seconds) | 370 | 98 | 356 | 362 | 120 | 523 | 143 | 195 | 146 |
| Seizure window (hours) | 7 | 9 | 4 | 24 | 16 | 11 | 21 | 35 | 54 |
| Status epilepticus | None | None | Yes | None | None | Yes | Yes | Yes | Yes |
| Number of seizures (n) | 3 | 41 | 17 | 22 | 81 | 23 | 112 | 101 | 136 |
| Seizure classification | | | | | | | | | |
| Electrographic-only seizures: n (%) | 0 (0) | 27 (66) | 8 (47) | 20 (91) | 77 (95) | 13 (57) | 77 (69) | 62 (61) | 121 (89) |
| Electrographic-only seizure burden: minutes (%) | 0 (0) | 28 (42) | 26 (25) | 129 (97) | 146 (90) | 74 (37) | 129 (49) | 244 (74) | 282 (85) |
| Electroclinical seizures: n (%) | 2 (66) | 10 (24) | 7 (41) | 1 (4.5) | 3 (3.7) | 9 (39) | 32 (29) | 35 (35) | 15 (11) |
| Electroclinical seizure burden: minutes (%) | 18 (95) | 30 (44) | 48 (48) | 3 (2) | 15 (9) | 108 (54) | 126 (47) | 80 (24) | 50 (15) |
| Clonic/subtle seizures: n | 2/0 | 0/10D | 5/2C | 1/0 | 3/0 | 0/9S | 17/15S | 16/19Y | 9/6M |
| Video obscured: n (%) | 1 (33) | 4 (10) | 2 (12) | 1 (4.5) | 1 (1.3) | 1 (4) | 3 (2) | 4 (4) | 0 (0) |
| Subtle seizures: C, cycling movements of the limbs; D, desaturations; M, mouthing and smacking of lips; S, sucking; Y, yawning. | | | | | | | | | |

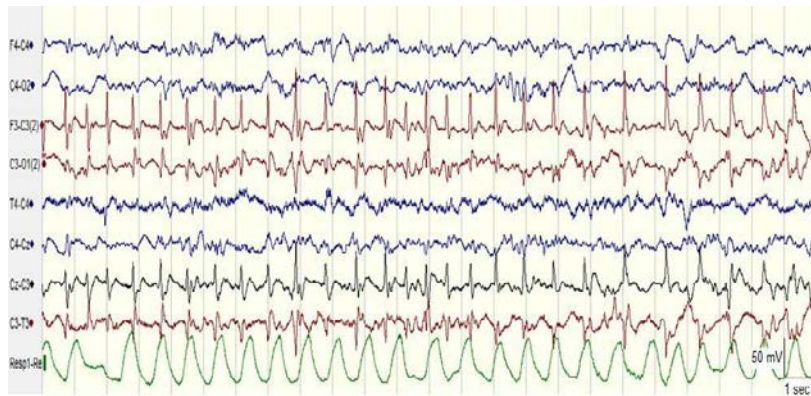
Figure 7.2 Background EEG pattern in a neonate (case 9) with a right middle cerebral artery infarction.



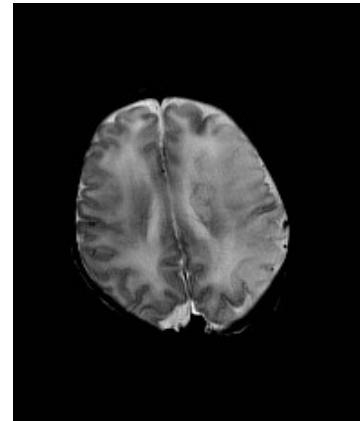
Note the mild voltage reduction over the right hemisphere on EEG (blue channels or even numbered channels) which is also evident on the aEEG with a wider band on the right in comparison to the left side (odd numbered channels). In addition, intermittent right-sided bursts of higher voltage sharpened theta activity are also evident. Some sleep cycling is also present over the left albeit disturbed but this is absent over the right side.

Figure 7.3 EEG in a neonate (case 6) with seizures arising from the left hemisphere.

a) EEG recording

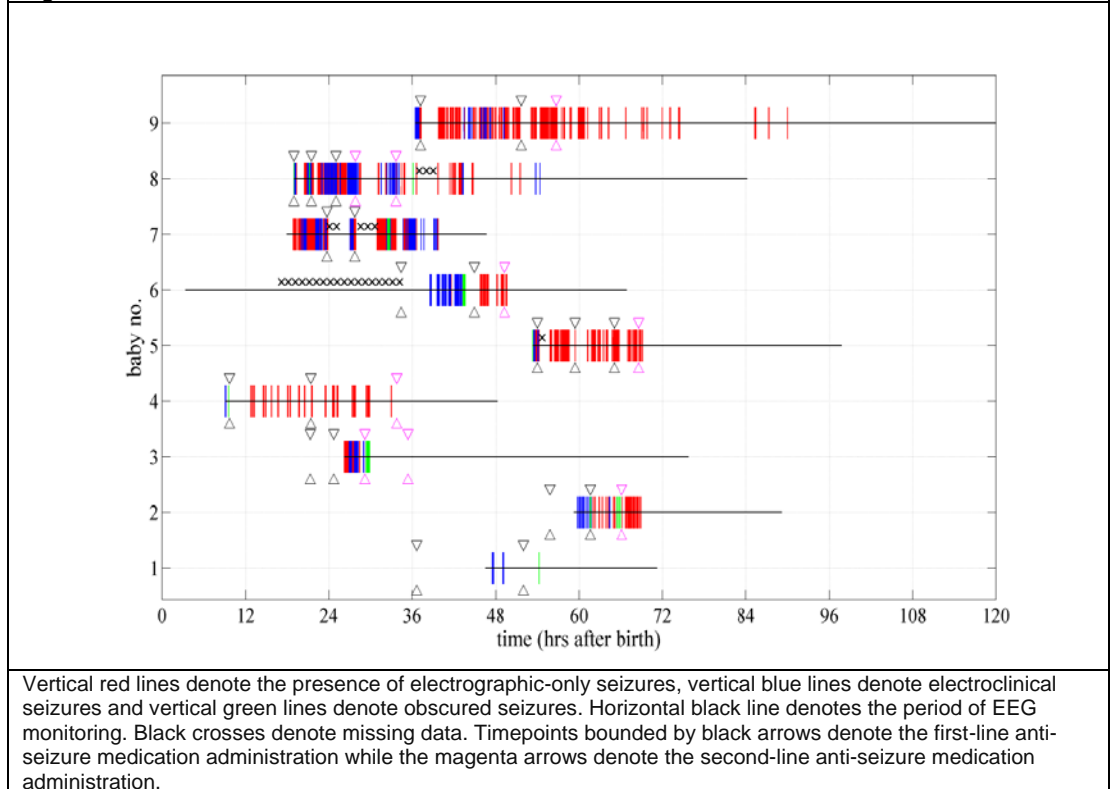


b) MRI imaging



Electrographic seizures arising from the left sided channels of the EEG (F3-C3, C3-O1, Cz-c3, C3-T3) corresponding to the frontal (F), central (C), occipital (O) and temporal (T) areas of the brain. Note the characteristic focal spike and wave discharges over the left hemisphere with phase reversal over the left central region (odd numbered channels). This is corresponding with a left middle cerebral artery infarction on cranial MRI. The sequence is an axial T2 turbo spin echo performed on day 7 of life.

Figure 7.4 Characteristics of seizures and anti-seizure medication administration in each neonate.



Of 536 electrographic seizures identified from multichannel EEG in this cohort of neonates with PAIS; 519 were classified (table 7.2). Accumulatively, there were more electrographic-only ($n=405$; 78%) than electroclinical seizures ($n=114$; 22%). Summary measures of each neonate showed that the median (IQR) electrographic-only was higher than electroclinical seizures [66 (52-90) vs 29 (8-40)%; $p=0.051$]. Subtle seizures were noted in six of nine neonates and manifested activities such as pedalling or cycling movements of the limbs, sucking or chewing movements.

Other occasional subtle seizures noted were hiccups and eye blinking episodes. When electroclinical seizures were subdivided, there were more subtle ($n=61$; 12%) than clonic seizures ($n=53$; 10%) [median (IQR) of subtle vs clonic seizures=12 (0-22) vs 7 (2-24)%; $p=0.553$]. The median seizure burden of electrographic-only was higher than electroclinical seizures [49 (31-88) vs 44 (12-51)%; $p=0.515$]. This is despite the significantly shorter median duration of electrographic-only when compared to electroclinical seizures [100 (55-173) vs 181 (95-359) seconds; $p<0.001$].

The temporal distribution of electrographic-only and electroclinical seizures with the administration of anti-seizure medication superimposed for each neonate are shown in figure 7.3. In four of nine neonates (cases 1, 2, 3 and 6), anti-seizure medication were administered prior to prolonged multichannel EEG monitoring, hence before the first electrographic seizure. All nine neonates with PAIS received first-line anti-seizure medication at age of 34 (20-46) hours while seven neonates received second-line anti-seizure medication at 48 (29-66) hours.

7.6 Discussion

This study has shown that in term neonates with stroke, the electrographic seizure burden was approximately as high as up to 80% of all seizures. There is a characteristic signature on the EEG which was identified clearly in all neonates with stroke. The background EEG generally revealed a marked suppression (>50% amplitude reduction) if the infarction was large (>66% estimated size of infarction) over the affected hemisphere. A characteristic unilateral theta bursts with intermixed sharp or spike waves were seen in all cases over the infarcted side. Sleep cycling was more disrupted over the infarcted side.

Seizures in neonates with PAIS appear to have a characteristic pattern and in all cases, focal sharp waves/spike-polyspike seizure discharges were seen at a frequency of 1 to 2 Hz over the area of infarction. The morphology of these seizures in stroke has been noted to be different from seizures arising from HIE (Lynch N et al., 2011). In this study cohort of neonates with MCA infarction, seizures were generally seen over the central region and phase reversal of spike and polyspike discharges were a common finding. This is the first study in detailing the characteristic electrographic seizure burden and to describe the characteristic EEG findings in a series of neonates with PAIS in the early postnatal period; these findings may prove very useful for early diagnosis of neonates with seizures.

Clinical diagnosis of PAIS

PAIS tends to be a clinical diagnosis when three important findings are present: no clear history of HIE, seizure onset beyond 12 hours after birth and focal seizures. In many instances when the affected cases are discussed retrospectively, subtle details are often missed; they usually revealed a slightly complicated antenatal history such as mild changes on the cardiotocogram or meconium stained delivery (Mercuri, 2001). Apgar scores and clinical history may be subjective. The use of the EEG is advocated as an adjunct to suggest the

early diagnosis of PAIS during the neonatal period when clinical suspicions are aroused.

Neonates with PAIS are usually noted to be non-encephalopathic (Cowan et al., 2003)(such as normal feeding, absence of abnormal tone or absence of a depressed level of alertness); however hypotonia, poor sucking reflex and irritability have been described (Miller, 2000). Clinical signs may not manifest if the motor cortical strip is not involved. All neonates in this study had some degree of MCA involvement and at some timepoints; a clinical correlate which can be often very subtle was evident. Subtle seizures in this cohort of neonates with PAIS involved mainly oral-buccal-lingual movements (four of six neonates); this is in line with other studies (Pinto and Giliberti, 2001; Volpe JJ, 2008).

In PAIS, autonomic dysfunction such as apnoeic spells (Fujimoto et al., 1992; Hoogstraate et al., 2009) has been reported in up to 36% of neonates (Sreenan et al., 2000); only one neonate in this study presented with apnoea before any anti-seizure medication administration. More studies are required to investigate and explain as to why EEG suppression tends to occur at the side of infarction in neonates. However, there have been several studies showing how apnoea can affect the electrical activity and hence the EEG in neonates with some degree of brain injury (Thoresen et al., 1996; Gavilanes et al., 2004; Low et al., 2012b)

In the newborn piglet model, hypoxic-ischaemic events induced by reducing fractional inspired oxygen to around 6% has been shown to generate a rapid suppression of EEG activity. Brain injury was only seen when the EEG amplitude remained suppressed for 23 minutes or more (Thoresen et al., 1996). In another study where one week old piglets were subjected to graded hypoxia, the EEG amplitude did not decline until oxygen saturation fell below 25% (Gavilanes et al., 2004). This is similar to episodes of EEG suppression observed in a neonate in our neonatal intensive care unit (Low et al., 2012b). Hypoxia in conjunction with bradycardia was responsible for the severe EEG suppression in the reported case. Bradycardia preceded complete EEG suppression and EEG amplitude did not become profoundly suppressed until oxygen saturation fell below 20%.

Other subtle seizures which have been previously described in neonates with stroke included eye blinking, vertical nystagmus and thumb adduction (Fujimoto et al., 1992), but multichannel EEG monitoring was not applied, thus the accuracy of these clinical signs is unknown. The results of this study support the suggestion for lower threshold in initiating EEG monitoring when there is any suspicion of unusual movements which may be seizures.

High seizure burden in neonates with PAIS

The pathophysiology of neonates with stroke differs from those with HIE. Although many studies have reported the incidence of seizures in neonates with stroke, they were mainly based on clinical observations rather than the multichannel EEG. Few studies have reported the seizure burden in neonates with HIE, but no studies have quantified the seizure burden in neonates with stroke. By quantifying the seizure burden in this vulnerable group of neonates, this will enlighten us our current status of the situation in the neonatal intensive care unit.

Although therapeutic hypothermia has been widely accepted as the current standard of care to treat neonates with HIE, this has not been advocated for neonates with stroke. We have shown that therapeutic hypothermia reduced seizure burden in term neonates with hypoxic-ischaemic encephalopathy (previously discussed in Chapter 6: the Cooling study). Perhaps, therapeutic hypothermia should be advocated for neonates with stroke to reduce the occurrence of seizures and hence the seizure burden in this group of neonates.

Use of anti-seizure medication in neonates with PAIS

The overall seizure burden was high in this current study; early, prolonged and continuous multichannel video-EEG monitoring showed that the number of seizures is higher than clinically apparent. Anti-seizure medications were administered when there was a clinical concern of seizures. The use of anti-seizure medication may have resulted in more electrographic-only seizures (Glykys et al., 2009); this study has shown 80% of the total seizure burden was ascribed to electrographic-only seizures. Anti-seizure medication has been shown to cause electroclinical dissociation of seizures (Boylan et al., 2002).

The high number of seizures uncovered in this group of neonates was surprising but reinforces the need for early, prolonged and continuous multichannel EEG monitoring in this group of neonates. In comparison, electroclinical dissociation of seizures has been reported to occur up to 28% of neonates with HIE; however this figure was based on aEEG findings in neonates above 32 weeks gestation and its association with anti-seizure medication administration was not described (Vasiljevic et al., 2012).

The studies by van Rooij *et al.* (van Rooij et al., 2010a) and Mercuri *et al.* (Mercuri et al., 1999) did not provide information on the electroclinical dissociation of seizures. Many of the previous studies reported the clinical response to anti-seizure medication without any EEG monitoring (Estan and Hope, 1997; Golomb et al., 2007; Rando et al., 2000). It is known that anti-seizure medication can be a sedative agent and lead to electroclinical uncoupling or dissociation of seizures (Boylan et al., 2002). Clinical seizures are therefore a poor indicator when it comes to assessing the response to anti-seizure medication; hence the true response of anti-seizure medication in seizure control in neonates with PAIS remains unknown.

This research study highlights that despite the use of anti-seizure medication, under tight EEG monitoring, there are still ongoing electrographic seizures in neonates with PAIS. Neonatologists should be aware of this when treating neonates with PAIS who are already treated with initial anti-seizure medication, particularly in the absence of EEG monitoring. This also explains why several neonates in this research study had many hours of repetitive seizures and were not treated with anti-seizure medication. This study is the first to demonstrate the high seizure burden in PAIS using continuous multichannel EEG monitoring and is thus of significant and practical clinical importance.

Monitoring seizures in neonates with PAIS

In neonatal stroke studies which used the aEEG (F3-P3 and F4-P4), some localized seizures may have been missed. The MCA is the most commonly involved artery for ischaemic infarction in term neonates (the posterior branch irrigates the occipital, temporal and posterior parietal areas, while the anterior branch irrigates the prefrontal, precentral, central and anterior parietal areas) (Govaert et al., 2000). Therefore, a comprehensive EEG electrode coverage of the scalp is required to ensure that all seizures are detected. This research study had

relied on monitoring using the multichannel EEG with a bipolar montage consisting of at least 8 channels to capture seizures; this has allowed a more accurate measurement of seizure burden to be obtained.

Comparing one-channel with the two-channel aEEG recordings in 34 neonates who had seizures due to unilateral brain injury, van Rooij *et al.* showed more varied seizures patterns, asymmetry in the background activity and a difference in sleep cycling on the ipsilateral side (van Rooij *et al.*, 2010a), however this study gave no specific analysis on a subgroup of neonates who had PAIS (n=5) or specifically those who had MCA involvement (n=3). Using a four-channel aEEG in 19 neonates with PAIS (6 neonates with asymmetrical and 2 with bilateral sharp waves/ spikes, 8 neonates with no seizures, 3 neonates had no aEEG recorded), Mercuri *et al.* showed that the presence of seizures accompanied by a normal background EEG was not related to abnormal outcome (Mercuri *et al.*, 1999); this indicates that both factors are poor predictors of outcome. Although this research study was not aimed to assess outcome, an abnormal background and the presences of seizures have a much higher prognostic value. Also, the study by Mercuri *et al.* had not assessed seizures as an independent factor in determining outcome (Mercuri *et al.*, 1999).

Multichannel EEG has been shown to be more accurate than the aEEG in detecting seizures. Our EEG findings based on multichannel EEG recordings are similar to studies by van Rooij *et al.* (van Rooij *et al.*, 2010a) and Mercuri *et al.* (Mercuri *et al.*, 1999) which used the aEEG, however we have provided more details on the characteristics of seizures early in the neonatal period in terms of seizure morphology and more detailed seizure characteristics in a cohort of neonates with PAIS.

To date, reported incidences of seizures in neonates with PAIS are mainly based on observation of neonatal behaviours (Golomb *et al.*, 2007; Kirton *et al.*, 2011), rather than on multichannel EEG which is the gold standard for accurate detection of neonatal seizures (Glass and Wirrell, 2009; Low *et al.*, 2012a; Murray *et al.*, 2008; Wusthoff *et al.*, 2011). Approximately 20% of neonatal seizures in term neonates are due to PAIS (Volpe JJ, 2008). Conversely, while neonatal seizures have been noted in 26% of neonates with PAIS (Rafay *et al.*, 2009), these numbers could be much higher if detection of seizures is based on early, prolonged and continuous multichannel EEG monitoring.

Most neurological presentations of neonates with PAIS occur in the first 72 hours of life. The age of first clinical seizure and first recorded EEG seizure [33 (17-42) and 36 (19-54) hours] were within 72 hours of age. In this study, EEG monitoring was initiated only after clinical seizures were observed. This is consistent with current practice in most neonatal units as (unlike HIE) there are no existing early indicators or biomarkers to identify neonates with PAIS, hence it is possible that neonates with PAIS and electrographic-only seizures may have been missed and escaped detection during early recording period. Early EEG monitoring may have a role in providing an early indicator of PAIS, as early EEG from three hours after delivery has been shown to display occasional focal sharp waves over the infarcted region which became more frequent, complex and of higher amplitude in quiet sleep (Walsh et al., 2011).

A limitation of this study is the small number of neonates with PAIS. In this cohort of neonates, all except one neonate (case 2) was captured when they presented with hemiconvulsions before discharge shortly after birth in the 2 neonatal units. This study only included neonates that presented with clear PAIS involving at most 2 arterial territories and who had continuous multichannel EEG monitoring as soon as possible after their presentation with seizures. While being monitored, these neonates with seizures showed asymmetrical characteristics on the EEG. In this period, other neonates would have presented but did not have continuous EEG monitoring undertaken.

It is difficult to diagnose all neonates with PAIS in the neonatal period as the majority of term neonates affected by PAIS are asymptomatic (Lynch and Nelson, 2001); appearing clinically well enough to be sent to the postnatal ward shortly after birth. In the 2 neonatal units, there is a policy of early maternal and neonatal discharge. Any neonate presenting with seizures after they were discharged would have been readmitted to regional paediatric hospitals, not the neonatal units. Even though the number of neonates with PAIS is small, the novelty here is having captured a number of neonates who had early and long duration of multichannel EEG monitoring.

7.7 Conclusion

Neonates with PAIS demonstrated distinctive features in the background EEG and morphology of seizures. These features were present and can be detected from very early in life hours after delivery. Detailed EEG analysis may prove very useful

for early diagnosis of PAIS. For the first time, this study has quantified the seizure burden in neonates with PAIS using multichannel video-EEG. About 80% of all seizures in neonates with PAIS will escape detection without early, prolonged and continuous multichannel EEG monitoring.

What this study adds?

- Using the multichannel video-EEG, this is the first study to report that in neonates with stroke, the background EEG shows asymmetry and suppression over the infarcted side; characteristic unilateral bursts of theta activity, sharp waves and spikes were present.
- Electrographic seizures in neonates with stroke have a particular focal sharp wave/spike-polyspike pattern and phase reversal is frequently present.
- Approximately 80% of the total seizure burden in term neonates with stroke is not recognizable without the use of continuous multichannel video-EEG monitoring.

Chapter 8

Characteristics of Electrographic Seizure Burden in Response to Phenobarbitone in Term Neonates

8.1 Abstract

Purpose To assess the effectiveness of phenobarbitone as first-line anti-seizure medication for neonatal seizures using continuous multichannel EEG monitoring.

Patients Neonates more than or equal to 37 weeks gestation, born between 2009 and 2011 in two hospitals.

Methods Electrographic seizures were annotated on the EEG recording by 2 experienced neonatal electroencephalographers. Instantaneous seizure burden (ISB) was defined as the accumulated duration of electrographic seizures within an hour of EEG monitoring and is expressed in minutes/ hour. In each neonate, the maximum ISB was used to assess the effectiveness of phenobarbitone and was calculated in the following time periods: a 1 hour period beginning 1 hour prior to each dose of phenobarbitone (T_{-1}), a 1 hour period beginning immediately after cessation of each phenobarbitone infusion (completed in 30 minutes) (T_{+1}) and the remaining duration of EEG monitoring beginning 1 hour after cessation of the last dose of phenobarbitone infusion (T_{+LP}).

Results Thirty-five neonates had electrographic seizures [hypoxic-ischaemic encephalopathy (n=20), stroke (n=8), benign seizures (n=2), intraparenchymal haemorrhage (n=2), subdural haemorrhage (n=1), meningitis (n=1) and seizure of unknown cause (n=1)]. EEG monitoring began at age 9 (5-28) hours, EEG duration was 69 (49-104) hours and the median (interquartile ranges) age of first EEG seizure was 19 (11-36) hours. Two of 35 neonates with electrographic-only seizures did not receive anti-seizure medication therapy.

Of the thirty-three neonates treated with phenobarbitone, 19 were treated concurrently with electrographic seizures. The maximum ISB was significantly reduced within 1 hour of phenobarbitone administration from T_{-1} to T_{+1} [mean difference (95% confidence interval): -14.0 (-20 to -8) minutes/hour; $p < 0.001$]. Seizures abated in 3 neonates while in 16 neonates, seizures returned to levels not significantly different to pre-treatment levels within 4 hours of first

phenobarbitone administration ($p=0.064$). Compared with 10 mg/kg doses, a subgroup analysis revealed that only phenobarbitone doses at 20 mg/kg resulted in a significant reduction in maximum ISB from T_{-1} to T_{+} ($p=0.004$). Phenobarbitone was more effective in the short-term if administered when seizure burden was low ($p=0.005$).

Conclusion Treatment with phenobarbitone has an immediate effect in reducing electrographic seizure burden in term neonates. This reduction however did not last, with seizures returned in the majority of neonates. Further research on the precise mechanistic action of phenobarbitone in the human neonatal brain is required if the clinical incentive is to abolish seizures in the developing neonatal brain.

8.2 Introduction

Since 1950 when phenobarbitone was discovered to chiefly act on the human brain, (Loscher and Rogawski, 2012) the use of anti-seizure medication to treat neonatal seizures has not changed significantly in clinical practice; this is in part due to the lack of randomized control trials in human neonates. In particular among neonatologists, this poses an uncertainty on the effectiveness of currently use mono/ polypharmacy medication such as phenobarbitone, lorazepam, phenytoin and midazolam to treat neonatal seizures. This form of treatment remains unchallenged despite evidence in animal studies which showed that phenobarbitone is effective as an anti-seizure medication in approximately only 50% of neonatal seizures (Painter et al., 1999).

Some authors have cautioned the use of phenobarbitone since it has been associated with dire long-term neurodevelopmental consequence due to its ability to reduce the growth and development of the neonatal brain (Bittigau et al., 2003). In many neonatal units today, neonates with hypoxic-ischaemic encephalopathy (HIE) who undergo therapeutic hypothermia will have a combined treatment with phenobarbitone as the initial treatment for seizures (Low et al., 2012a). The evidence of effectiveness of phenobarbitone from the combination of treatment with therapeutic hypothermia or with other anti-seizure medication has yet to be further studied and evaluated.

To date among neonatologists, further related controversy centres on the issue of timing for treating neonatal seizures. Most neonatologists will instigate treatment with anti-seizure medication when there are observable clinical seizures at the cotside. However, most neonatal seizures are subclinical seizures (Malone et al., 2009; Murray et al., 2008; Yap et al., 2009). Subclinical seizures are not recognizable without the use of the multichannel-EEG monitoring. With the use of the amplitude integrated EEG (aEEG), artefacts may be misinterpreted as seizures, leading to unnecessary exposure of the neonatal brain to the use of anti-seizure medication and its potential side effects. Therefore, accurate monitoring of the response of seizures to anti-seizure medication warrants the use of the multichannel EEG.

What is already known on this topic?

- To date, phenobarbitone remains the most popular anti-seizure medication used by clinicians to treat neonatal seizures.
- Approximately 40 to 50% of neonates with seizures will respond to phenobarbitone treatment.

8.3 Aims

Hypothesis: Administered doses of phenobarbitone lower than 20 mg/kg are not as effective as at 20 mg/kg. The current treatment strategy clearly questions the effectiveness of phenobarbitone in terms of dosage and the timing of administration.

Study aim: To determine the characteristics of seizures in neonates treated with phenobarbitone (the Phenobarbitone study). As part of the process in assessing the effectiveness of current anti-seizure medication treatment strategy, this study aimed to determine the effect of phenobarbitone on neonatal seizures specifically in relation to the degree of reduction in electrographic seizure burden in term neonates during continuous and prolonged multichannel video-EEG monitoring.

8.4 Methods

The methodology involved in this study is described in detail in Chapter 5 (Methodology).

Instantaneous seizure burden (ISB) is defined as the accumulated seizure duration within a 1 hour window which was shifted across the EEG monitoring period with a 1 minute interval to generate a time series. The ISB provides a continuous summary of an hour of seizure activity, thereby reflecting a measurement of the intensity of seizures over the time course of seizures in a neonate; it is defined as:

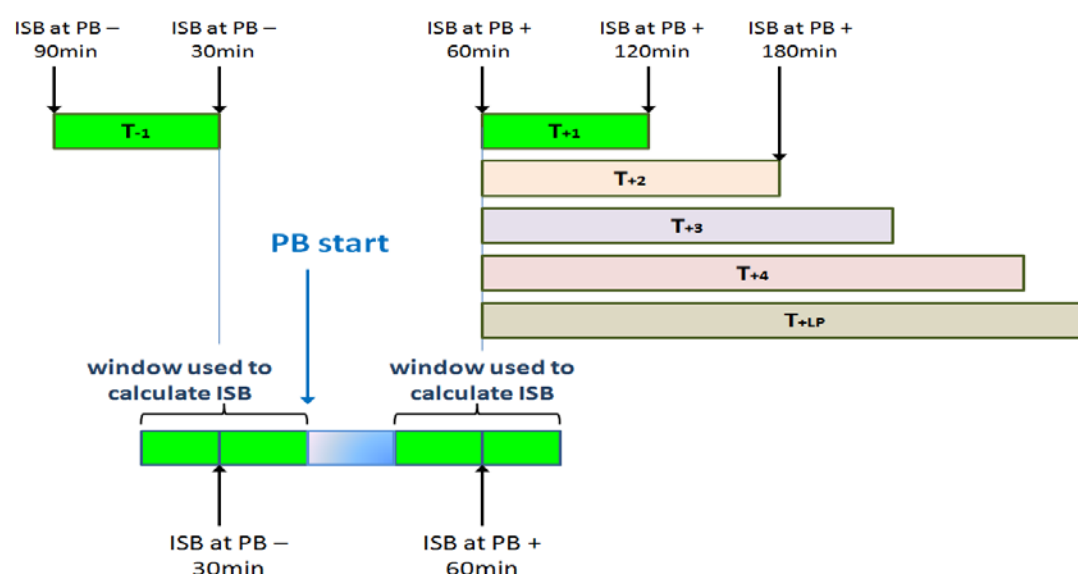
$$ISB(t) = \int_{t-T_w}^{t+T_w} s(t_1) dt_1$$

where $t = t_1$ is time, T_w is 30 minutes and $s(t)$ is the seizure annotation based on visual interpretation of the EEG.

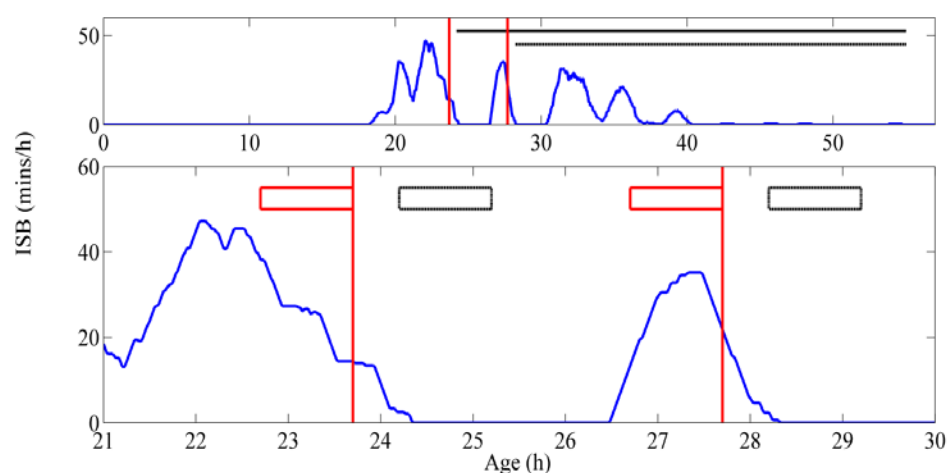
In each neonate, the maximum ISB within a pre-defined time period was used to assess the effectiveness of phenobarbitone and was calculated in the following time periods: a 1 hour period beginning 1 hour prior to each dose of phenobarbitone (T_{-1}); a 1, 2, 3, 4, 5 until 8 hour period beginning immediately after cessation of each phenobarbitone infusion completed in 30 minutes (T_{+1} , T_{+2} , T_{+3} , T_{+4} , T_{+5} until T_{+8} respectively) and the remaining duration of EEG monitoring beginning 1 hour after cessation of the last dose of phenobarbitone infusion (T_{+LP}) when there was a seizure offset (figure 8.1).

Figure 8.1 The maximum instantaneous seizure burden (ISB)

A. The instantaneous seizure burden (ISB) was calculated at a 1 hour period beginning 1 hour (T_{-1}) before, at a 1 (T_{+1}), 2 (T_{+2}), 3 (T_{+3}), 4 (T_{+4}) hour period after each dose of phenobarbitone (PB) and the remaining period beginning immediately after cessation of each last phenobarbitone infusion (T_{+LP}).



B. An example plot of the ISB over time (blue line) for a single neonate with seizures overlaid with the time periods used to assess the effectiveness of phenobarbitone. The upper plot is the complete seizure time course for the neonate with T_{+LP} (black horizontal lines) shown for each phenobarbitone dose (red vertical lines). The lower plot is the magnified version of the upper plot with T_{-1} (red boxes) and T_{+1} (black boxes) shown for each phenobarbitone dose (red vertical lines). Note some smoothing is apparent in the ISB as both future and past values are used to estimate the ISB and a 30 minute delay is taken into account for phenobarbitone infusion. The maximum ISB within the time period of interest is used to assess the effectiveness of phenobarbitone. There is a clear reduction in maximum ISB between T_{-1} and T_{+1} following the administration of each phenobarbitone dose and these seizures returned within T_{+LP} .



An **effective dose** was defined as a dose which resulted in a reduction of the maximum ISB to an absolute zero during T_{+1} while an **ineffective dose** was defined as a dose which resulted in a reduction of the maximum ISB, but not to zero during T_{+1} . A **non-effective dose** was defined as a dose which resulted in an increase of the maximum ISB during T_{+1} .

Statistical analysis: Continuous variables were described using medians and interquartile ranges (IQR) and categorical variables using frequencies. For each neonate, differences between ISB pre- and maximum ISB for each period (T_{+1} , T_{+2} until T_{+LP}) post-phenobarbitone administration were calculated. Linear mixed models with a neonate-level random effect were used to account for possible correlations among observations from the same neonate (more than one dose of phenobarbitone per neonate). For comparisons between groups, group was included as a fixed effect in the linear mixed model.

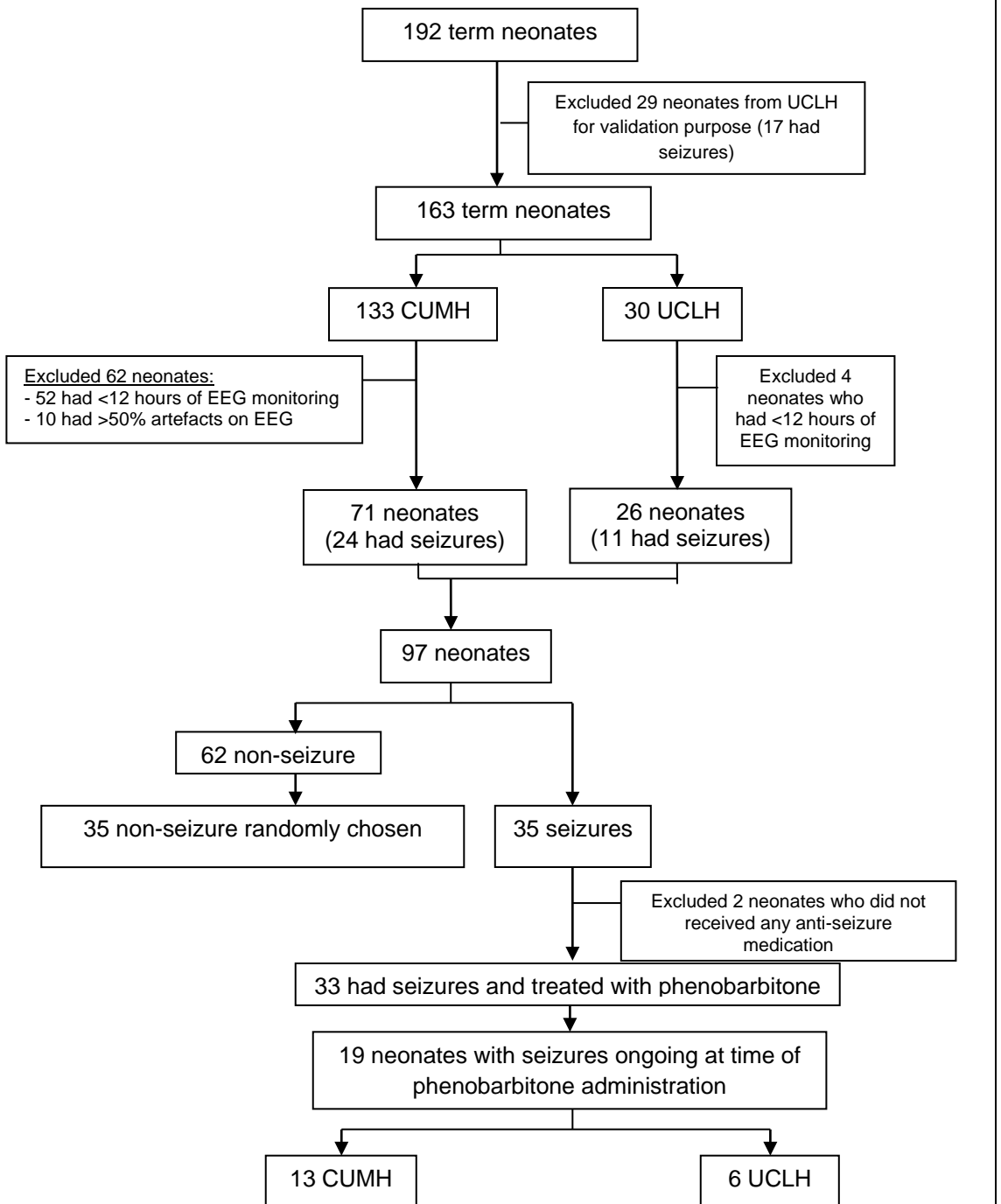
Results based on linear mixed models were presented as mean [95% confidence interval (CI)]. The following comparisons were also performed: comparison between ISB pre- and ISB post 1 hour (T_{-1} vs T_{+1}) by dosage, accumulated dosage and comparison of seizure burden at the time of phenobarbitone administration between effective and ineffective doses (analysis restricted to 20 mg/kg as there were no effective doses at 10 mg/kg). All statistical analyses were performed in SAS 9.3 (SAS Institute Inc., Cary, NC, USA) and a p value <0.05 was considered to be statistically significant.

8.5 Results

During the study period from 2009 to 2011, thirty-five neonates with electrographic seizures were identified (figure 8.2). The median (interquartile ranges) age when EEG monitoring began was 9.1 (4.7-28.1) hours, EEG duration was 69.0 (49.4-103.9) hours and the age of first EEG seizure was 19.0 (11.5-35.8) hours. Aetiologies for seizures included hypoxic-ischaemic encephalopathy (HIE) (n=20), stroke (n=8), benign seizures (n=2), intraparenchymal haemorrhage (n=2), subdural haemorrhage (n=1), meningitis (n=1) and seizure of unknown cause (n=1).

Figure 8.2 Flow diagram on the recruitment timeline for the Phenobarbitone study

Overall timeline: Jan 2009- June 2011



CUMH: Cork University Maternity Hospital, Ireland; UCLH: University College London Hospital, United Kingdom.

Table 8.1 and 8.2 list the clinical demographics, seizure burden, and the sequence in which anti-seizure medication were administered in neonates with HIE (5 of 20 neonates did not received therapeutic hypothermia) and due to other diagnoses respectively. Two of 35 neonates with electrographic-only seizures did not receive anti-seizure medication. A schematic diagram of the analysis of phenobarbitone administration with respect to the timing of seizures is depicted in figure 8.3.

Table 8.1 Individual characteristics of the first 19 neonates with electrographic seizures who were suitable for study analysis

| Case | Diagnosis | Cooling duration (age in hours) | Recorded seizure burden (mins) | Seizure number (n) | Mean seizure burden (secs) | Age of first anti-seizure medication (hours) | Age of EEG monitoring (hours) | Age of first EEG seizure (hours) | Duration of EEG monitoring (hours) | Order of anti-seizure medication given during EEG (mg/kg) |
|--------------------|---|------------------------------------|-----------------------------------|-----------------------|-------------------------------|---|----------------------------------|-------------------------------------|---------------------------------------|--|
| 1 | Arterial ischaemic stroke-LMCA | Non-cooled | 46 | 22 | 126 | 55.8 | 59.2 | 60 | 69 | PB(20, 10),PT |
| 2 ^E | HIE 2 | 72 (2-74) | 58 | 2 | 1741 | 9.9 | 4 | 8 | 77.12 | PB (20) |
| 3 | HIE 2 | 72 (2.5-74.5) | 44 | 21 | 127 | 11 | 4.7 | 11 | 78.9 | PB (20, 10) |
| 4 ^E | HIE 2 | 72 (2-74) | 119 | 18 | 396 | 15.6 | 2.7 | 13 | 101.25 | PB (20) |
| 5 | Multiple infarctions | Non-cooled | 12 | 4 | 183 | 115.2 | 113 | 113 | 14.87 | PB(20) |
| 6 ^E | HIE 2 | Non-cooled | 37 | 1 | 2207 | 7.3 | 2.9 | 7 | 60.92 | PB (20) |
| 7 ^E | HIE 3 | 65 (0.8-66) | 198 | 49 | 243 | 17.1 | 2.8 | 17 | 88.23 | PB (20, 20), PT |
| 8 | HIE 3 | 91 (2.1-93.1) | 397 | 296 | 80 | 15.1 | 6.8 | 12 | 127.02 | PB (20, 10, 10), MZ |
| 9 | Unknown | Non-cooled | 25 | 8 | 185 | 30.5 | 33.9 | 34 | 64.83 | PB (20, 20), PT |
| 10 ^E | HIE 3 | 22 (2.5-24.5) | 1404 | 266 | 317 | 9.7 | 4.4 | 9 | 172.37 | PB (20, 10, 10) |
| 11 ^R | HIE 3 | 72 (6-78) | 225 | 198 | 68 | 56.6 | 4.8 | 43 | 122.15 | PB (10,10,20), PT,CL |
| 12 | Arterial ischaemic stroke-LMCA and RMCA | Non-cooled | 133 | 22 | 362 | 9.7 | 9.1 | 9 | 38.92 | PB(20, 10), PT |
| 13 | Multiple infarctions | Non-cooled | 97 | 25 | 234 | 18.8 | 17.2 | 17.7 | 45.07 | PB(20, 20) |
| 14 ^{E, S} | HIE 3 | Non-cooled | 637 | 271 | 141 | 18.2 | 16.8 | 17 | 110.73 | PB (20, 20) |
| 15 ^E | Arterial ischaemic stroke-LMCA and LPCA | Non-cooled | 362 | 112 | 194 | 19 | 18.9 | 19 | 62.68 | PB(20, 10, 10), PT |
| 16 | HIE 2 | Non-cooled | 149 | 76 | 117 | 29 | 28 | 28 | 53.63 | PB (20, 10), MZ |
| 17 ^E | Viral encephalitis | Non-cooled | 80 | 28 | 171 | 55.5 | 57.9 | 58 | 103.93 | PB(20, 20) |
| 18 ^E | Arterial ischaemic stroke-RMCA | Non-cooled | 332 | 136 | 146 | 37.2 | 36.4 | 36 | 228.9 | PB(20, 20), MZ |
| 19 | Benign non-familial seizures | Non-cooled | 4 | 5 | 43 | 123 | 119.4 | 121 | 42.63 | PB (20), PY |

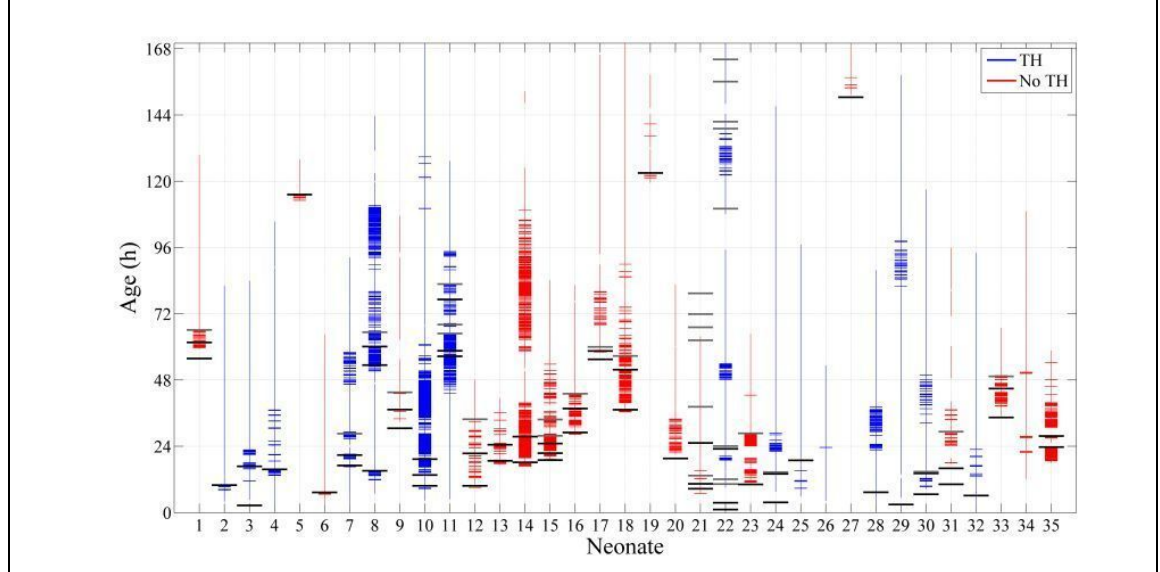
CL: Clonazepam; E: neonates with status epilepticus; LMCA: left middle cerebral artery; LPCA: left posterior cerebral artery; LV: Levetiracetam; MZ: Midazolam; PB: Phenobarbitone; PT: Phenytoin; PY: pyridoxine; R: neonates with EEG seizures following discontinuation of cooling; RMCA: right middle cerebral artery. S= neonates who were already seizing at the time when EEG record was commenced. Neonates involved in the Cooling study. Neonates involved in the Stroke study.

Table 8.2 Individual characteristics of the remaining 16 neonates with electrographic seizures who were excluded for study analysis.

| Case | Diagnosis | Cooling duration (age in hours) | Recorded seizure burden (mins) | Seizure number (n) | Mean seizure burden (secs) | Age of first anti-seizure medication (hours) | Age of EEG monitoring (hours) | Age of first EEG seizure (hours) | Duration of EEG monitoring (hours) | Order of anti-seizure medication given during EEG (mg/kg) | Comment |
|-----------------|--------------------------------------|---------------------------------|--------------------------------|--------------------|----------------------------|--|-------------------------------|----------------------------------|------------------------------------|---|--|
| 20 | Non-traumatic Intraparenchymal bleed | Non-cooled | 98 | 36 | 163 | 19.6 | 21.1 | 22 | 61.02 | PB(20) | One and only PB dose given before EEG commenced |
| 21 | Subdural haemorrhage | Non-cooled | 7 | 5 | 81 | 8.6 | 6.8 | 7 | 56.53 | PB (20, 10, 10), PT, CL, LV, PY | All 3 PB doses given during EEG monitoring but all not given during EEG seizures |
| 22 | HIE 3 | 72 (2-74) | 67 | 89 | 45 | 1.1 | 9.1 | 19 | 159.65 | PB (20, 10, 10), PT, CL, LV | First 2 PB doses given before EEG commenced and third PB dose given during EEG but not during EEG seizures |
| 23 ^S | HIE 2 | Non-cooled | 213 | 70 | 182 | 10.2 | 10.8 | 11 | 49.42 | PB (20) | One and only PB dose given before EEG commenced |
| 24 ^E | HIE 3 | 72 (0.66-72.7) | 27 | 19 | 86 | 3.7 | 7.6 | 22 | 138.48 | PB (20, 10), MZ | First PB given before EEG commenced, second PB dose given during EEG but not during EEG seizures |
| 25 | HIE 2 | 72 (4.8-76.8) | 24 | 4 | 359 | 18.9 | 5.8 | 9 | 91.22 | PB (20) | One and only dose of PB given during EEG but not during EEG seizures |
| 26 | HIE 2 | 11 (1.8-12.8) | 2 | 1 | 125 | None | 4.3 | 24 | 48.98 | None | No anti-seizure medication given |
| 27 | Benign familial neonatal seizures | Non-cooled | 2 | 4 | 28 | 150.4 | 151.3 | 153 | 33.45 | PB (20) | One and only PB dose given before EEG commenced |
| 28 | HIE 2 | 72 (3-75) | 78 | 54 | 87 | 7.3 | 3.3 | 23 | 84.25 | PB (20) | One and only PB dose given during EEG but not during EEG seizure |
| 29 ^R | HIE 2 | 72 (2-74) & 24 (122-136) | 22 | 26 | 51 | 2.9 | 5.5 | 81 | 54.97 | PB (20) | One and only dose of PB given before EEG commenced |
| 30 ^E | HIE 2 | 72 (5.4-77.4) | 82 | 28 | 176 | 6.6 | 8.5 | 9 | 108.23 | PB (20, 20), MZ | First PB given before EEG commenced, second PB dose given during EEG but not during EEG seizures |
| 31 ^E | Traumatic Intraparenchymal bleed | Non-cooled | 142 | 9 | 944 | 10.3 | 17.7 | 18 | 73.27 | PB (20, 10), PT | All 2 doses of PB given before EEG commenced |
| 32 | HIE 3 | 72 (0.25-72.3) | 24 | 9 | 161 | 6.2 | 1.6 | 13 | 92.28 | PB (20) | One and only PB dose given during EEG but not during EEG seizure |
| 33 ^E | Arterial ischaemic stroke-LMCA | Non-cooled | 207 | 22 | 564 | 34.4 | 3.3 | 39 | 45.63 | PB(20, 10), PT | First PB given before EEG commenced, second PB given when there is no EEG seizure |
| 34 | HIE 2 | Non-cooled | 51 | 7 | 438 | None | 12.1 | 22 | 97.08 | None | No anti-seizure medication given |
| 35 ^E | Arterial haemorrhagic stroke-LMCA | Non-cooled | 280 | 120 | 140 | 23.7 | 17.9 | 19 | 51.82 | PB(20, 20) | Neonates suitable for study analysis |

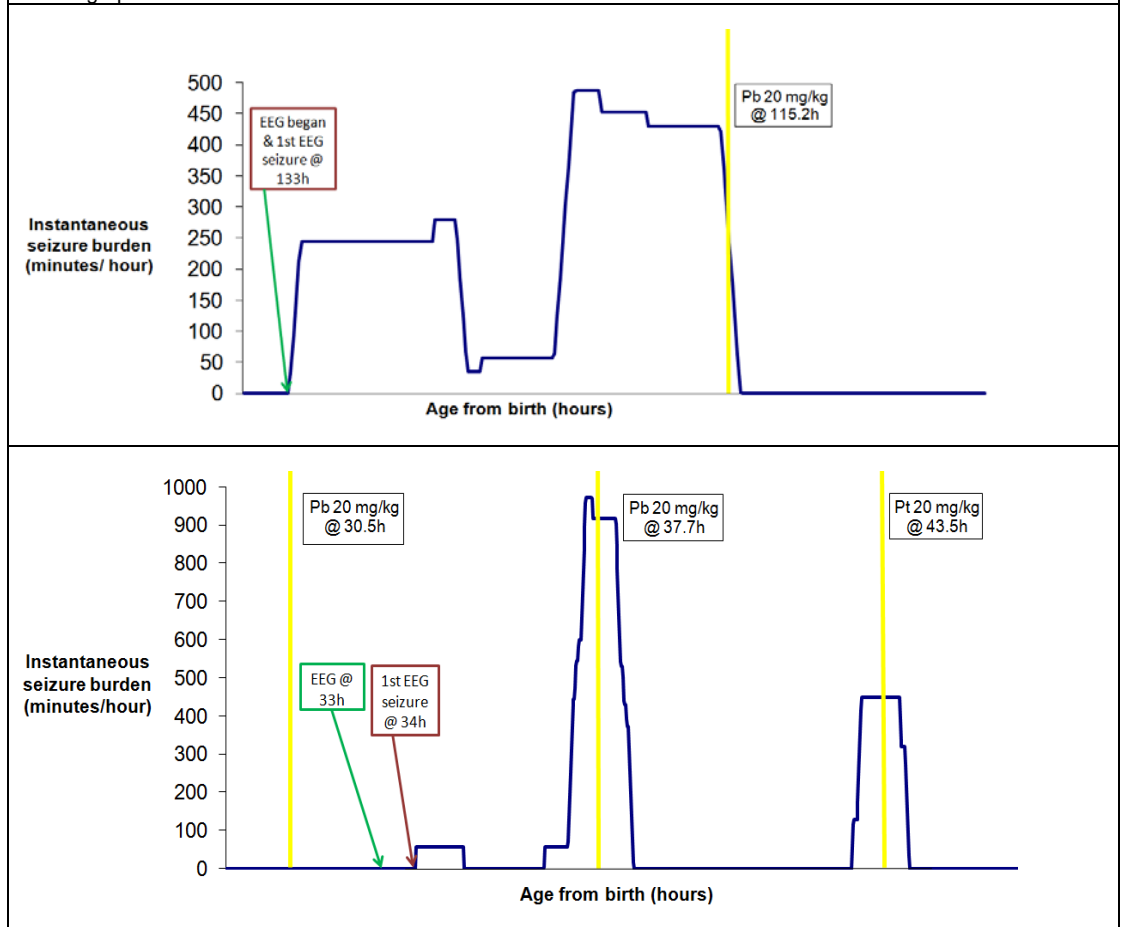
CL: Clonazepam; E: neonates with status epilepticus; LMCA: left middle cerebral artery; LPCA: left posterior cerebral artery; LV: Levetiracetam; MZ: Midazolam; PB: Phenobarbitone; PT: Phenytoin; PY: pyridoxine; R: neonates with EEG seizures following discontinuation of cooling; RMCA: right middle cerebral artery. S= neonates who were already seizing at the time when EEG record was commenced. [Neonates involved in the Cooling study.](#)
[Neonates involved in the Stroke study.](#)

Figure 8.3 A schematic diagram of ongoing electrographic seizures in all 35 neonates



The first 19 neonates were included for the study analysis. Clustered horizontal lines denote ongoing electrographic seizures in neonates who received therapeutic hypothermia (blue lines) and in neonates who did not received therapeutic hypothermia (red lines). Black horizontal bars denote phenobarbitone administration while grey horizontal bars denote second-line anti-seizure medication administration. Single vertical blue or red lines denote the duration of EEG monitoring. TH: therapeutic hypothermia.

Figure 8.4 A diagram showing the sequence of anti-seizure medication given in neonates with ongoing electrographic seizures



Two of 35 neonates with electrographic-only seizures did not receive any anti-seizure medication; case 26 was noted to have only 1 short seizure (2 minutes at about 0400 hours of the day), while case 34 was ventilated and sedated with no further clinical signs noted (seizure burden: 51 minutes, 7 number of seizures, mean seizure burden: 438 seconds) (table 8.1). Of the thirty-three neonates treated with phenobarbitone, 5 neonates (cases 20, 23, 27, 29, 31) had all their phenobarbitone doses administered shortly after clinical seizures were observed but before EEG monitoring commenced.

Case 29 had seizures during the rewarming period [not noticed for several hours on Saturday and Sunday (0800-1100)]; therapeutic hypothermia was recommenced soon after and seizures abated without further anti-seizure medication administration. Case 29 had a seizure burden of 22 minutes, 26 seizure events and the mean seizure burden was 51 seconds. Case 23 was a neonate admitted from the postnatal ward who presented with right sided jerking movements for which phenobarbitone was administered. The seizure burden in case 23 was 213 minutes, 70 number of seizures and mean seizure burden was 182 seconds. Case 23 had some recurring electrographic-only seizures (on Friday 0500-1430 hour); there were no clinical seizures to alarm the nursing or medical personnel. The EEG background pattern was supportive for the diagnosis of HIE but because the presentation was beyond the therapeutic window (>6 hours), therapeutic hypothermia was not administered.

Case 27 was also a neonate admitted from the postnatal ward who appeared clinically well with benign familial neonatal seizures; no further anti-seizure medication were given after the first dose of phenobarbitone (table 8.3). The seizure burden in case 27 was 2 minutes, 4 seizure events and the mean seizure burden was 28 seconds. Some electrographic-only seizures were noted during off-call hours (Tuesday 0007-0400), during which there was no neurophysiologist reporting on-call service.

Table 8.3 Reasons for neonates to be excluded for this study

| Case | Status | Reasons | Comments |
|------|----------|---|----------|
| 20 | Excluded | One and only PB dose given before EEG commenced | B |
| 21 | Excluded | 3 PB doses given during EEG monitoring but all not given during EEG seizures | N |
| 22 | Excluded | First 2 PB doses given before EEG commenced, 3rd PB dose given during EEG but not during EEG seizures | N |
| 23 | Excluded | One and only PB dose given before EEG commenced | B |
| 24 | Excluded | First PB given before EEG commenced, 2nd PB dose given during EEG but not during EEG seizures | N |
| 25 | Excluded | One and only dose of PB given during EEG but not during EEG seizures | N |
| 26 | | No anti-seizure medication given | |
| 27 | Excluded | One and only PB dose given before EEG commenced | B |
| 28 | Excluded | One and only PB dose given during EEG but not during EEG seizures | N |
| 29 | Excluded | One and only dose of PB given before EEG commenced | B |
| 30 | Excluded | First PB given before EEG commenced, 2nd PB dose given during EEG but not during EEG seizures | N |
| 31 | Excluded | All 2 doses of PB given before EEG commenced | B |
| 32 | Excluded | One and only PB dose given during EEG but not during EEG seizures | N |
| 33 | Excluded | 1st PB given before EEG commenced, 2nd PB given when there is no EEG seizures | N |
| 34 | | No anti-seizure medication given | |
| 35 | Excluded | No EEG monitoring after PB was administered | |

B: neonates excluded strictly because phenobarbitone (PB) given before EEG monitoring starts (n=5). N: neonates had either first PB dose given before or during EEG monitoring but were excluded because all PB were administered when there were no EEG seizures (n=9).

Cases 20 and 35 were neonates with focal brain lesions who presented with focal seizures; phenobarbitone was administered in the postnatal ward before admission to the NICU for continuous EEG monitoring. The seizure burden in case 20 was 98 minutes, 36 seizure events and the mean seizure burden was 163 seconds. There were no further clinical signs noted in case 20 during off-call hours (Saturday 0000-1200). The seizures burden in case 35 was 280 minutes, 120 number of seizures and the mean seizure burden was 140 seconds; electrographic-only seizures noted during off-call hours (Friday 0230-0730, 1030-1130, 1400-2330 and Saturday 0230-1400). Seizures were not treated in these 5 neonates after the first dose of phenobarbitone because all seizures were electrographic-only and there were no clinical or aEEG sentinels to alert the medical personnel to instigate treatment. The seizure burden in the 5 neonates (cases 20, 23, 27, 29, 31) was 477 minutes with 145 seizure events and the mean seizure burden 1368 seconds).

One neonate did not have EEG monitoring after phenobarbitone doses were administered case 35. Of the remaining 28 neonates who received at least one dose of phenobarbitone during EEG monitoring, 9 neonates had phenobarbitone doses administered when there were no ongoing electrographic seizures; they were treated for suspected ongoing clinical seizures.

In this cohort, some ongoing electrographic seizures were not treated; they typically emerged during weekends or past-midnight when there was no neurophysiologist available to interpret the multichannel EEG. In addition, the nursing and medical personnel had no clinical concerns about seizures despite continuous aEEG and EEG cotside display. Therefore, it was only possible to measure the true effectiveness of phenobarbitone treatment in the remaining 19 neonates who were treated concurrently with electrographic seizures; they form our study group: 10 neonates had HIE (7 cooled, 6 status epilepticus) and 9 neonates had other diagnoses (3 status epilepticus) (table 8.4). In total, 37 doses of phenobarbitone were given to these 19 neonates during EEG monitoring. Of these, 31 doses were administered when electrographic seizures were ongoing; 14, 13 and 4 doses were administered as first, second and third dose of phenobarbitone respectively.

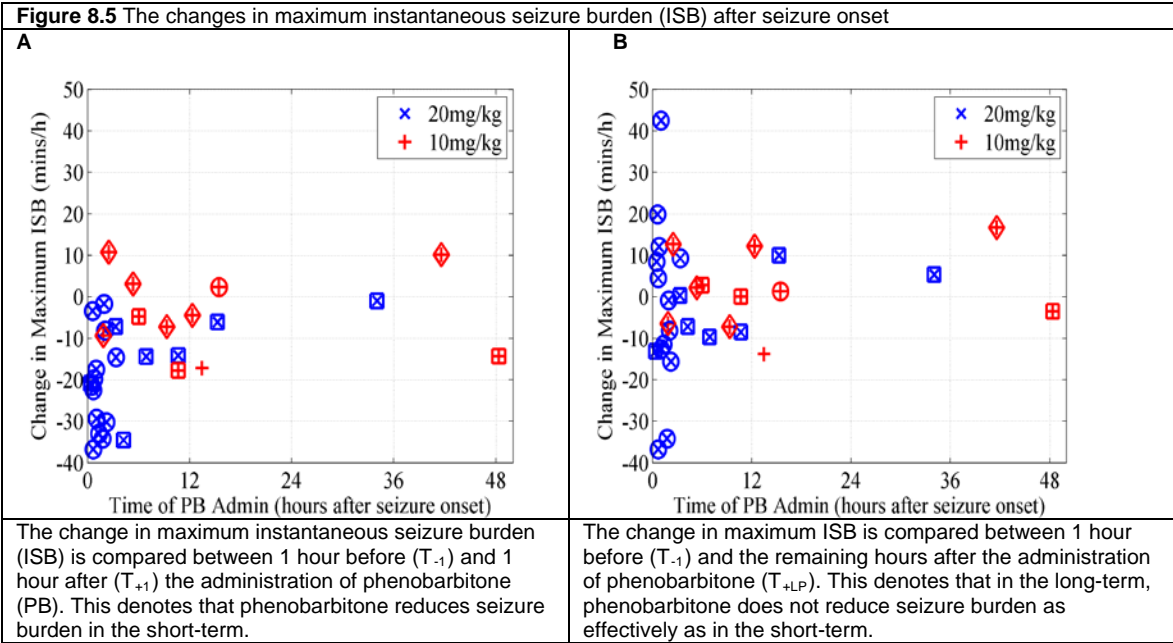
Table 8.4 Summary characteristics of the 19 neonates chosen for study analysis

| | |
|--|---------------|
| Age at EEG monitoring (hours) | 17 (4-36) |
| Age of first EEG seizure (hours) | 18 (11-41) |
| Duration of EEG monitoring (hours) | 78 (56-109) |
| Summary of seizure burden | |
| Recorded seizure burden (minutes) | 119 (45-305) |
| Seizure number (n) | 25 (11-130) |
| Mean seizure number (seconds) | 183 (126-298) |
| Status epilepticus | 9 |
| Received therapeutic hypothermia | 7 |
| Age of first anti-seizure medication (hours) | 19 (11-51) |
| Clinical diagnosis | |
| HIE 2 | 5 |
| HIE 3 | 5 |
| Multiple infarction | 2 |
| Focal arterial infarction | 2 |
| Bifocal arterial infarction | 2 |
| Viral encephalitis | 1 |
| Unknown cause | 1 |
| Benign non-familial seizures | 1 |

Data are expressed as n or median (IQR)

Phenobarbitone is more effective when given sooner after seizure onset (figure 8.5A), but this effect wears off in the long-term (figure 8.5B). There were 9, 8 and 2 neonates who had 1, 2 and 3 doses of phenobarbitone analyzed respectively. The median (IQR) time between electrographic seizure onset and first analyzed dose was 1.8 (0.7-2.4) hours. When a neonate received more than 1 dose, the time between doses of phenobarbitone was 9.0 (4.8-13.1) hours (12 of 29 doses). Only 10 of 19 neonates received a second-line anti-seizure medication; the time from the last dose of phenobarbitone to the first dose of second-line anti-seizure medication

was 5.1 (2.8-6.3) hours. One neonate received a second-line anti-seizure medication 12 hours before the last dose of phenobarbitone was administered.



Comparison between timepoints

In these 19 neonates, the maximum ISB was significantly reduced 1 hour immediately after the administration of phenobarbitone from a mean difference (95% CI) of -14.0 (-19.6 to -8.5) minutes/hour; $p<0.001$ (figure 8.5A).

No seizures were observed during T_{+1} in 13 of 19 neonates (13 of 31 doses). In 3 neonates, seizures were permanently abolished after one dose of phenobarbitone (20 mg/kg) administration. In the remainder, this overall reduction was not maintained permanently as the maximum ISB during T_{+LP} increased when comparing maximum ISB in T_{-1} to T_{+LP} [mean difference (95% CI) of -2.3 (-9.2 to 4.5) minutes/hour; $p=0.481$ (figure 8.5B)]. The reduction in maximum ISB due to phenobarbitone administration was not significant by T_{+4} ; that is within 4 hours of first phenobarbitone administration (table 8.5).

Table 8.5 Results of linear mixed models for maximum instantaneous seizure burden (ISB) post and pre-1 hour of phenobarbitone administration from 31 observations at each timepoint across the 19 neonates.

| ISB: Post-pre phenobarbitone administration | Difference in means (95% confidence interval) in minutes/hour | p value |
|---|---|--------------|
| Post: 1 hour | -14.04 (-19.60 to -8.48) | <0.001 |
| Post: 2 hours | -9.48 (-15.05 to -3.91) | 0.003 |
| Post: 3 hours | -7.53 (-13.34 to -1.73) | 0.016 |
| Post: 4 hours | -5.38 (-11.15 to 0.39) | 0.064 |
| Post: 5 hours | -4.89 (-10.70 to 0.93) | 0.089 |
| Post: 6 hours | -4.99 (-10.88 to 0.91) | 0.090 |
| Post: 7 hours | -3.39 (-9.78 to 2.99) | 0.268 |
| Post: 8 hours | -3.29 (-9.78 to 3.21) | 0.292 |
| Post: 9, 10, 11 hours | -2.92 (-9.31 to 3.46) | 0.338 |
| Post: 12 hours | -2.92 (-9.31 to 3.47) | 0.338 |
| Until T _{+LP} | -2.33 (-9.20 to 4.54) | 0.481 |

The maximum ISB was reduced to 0 during T₊₁ for 13 (10 as first doses of phenobarbitone at 20 mg/kg; 3 as second doses of phenobarbitone at 20 mg/kg) of 31 analyzed phenobarbitone doses given to 13 neonates (table 8.6). The maximum ISB was reduced (but not to 0) during T₊₁ for 14 of 31 doses given to 11 neonates; seven of 14 doses were 10 mg/kg (1 as first dose, 3 as second dose and 3 as third dose) and seven were at 20 mg/kg (3 as first dose, 3 as second dose and 1 as third dose). For 4 (all second doses of phenobarbitone at 10 mg/kg) of 31 doses in 4 neonates, there was an increase in maximum ISB during T₊₁.

Table 8.6 Details on the administration of phenobarbitone (PB) and the maximum instantaneous seizure burden in the 19 neonates

| Case | PB dose (mg/kg) in sequence | PB dose (mg/kg) analyzed | Accumulated dose (mg/kg) | Maximum ISB before PB administration (mins/hr) | Maximum ISB after PB administration (mins/hr) | % reduction of maximum ISB between T ₊₁ and T. | Maximum ISB after PB administration (mins/hr) | | | | | | | | | | | | Until seizure offset |
|------|-----------------------------|--------------------------|--------------------------|--|---|---|---|------|------|------|------|------|------|------|------|------|------|------|----------------------|
| | | | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| | | | | | | Δ | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| 1 | 20*, 10 | 10 | 30 | 13.9 | 4.5 | 67.5 | 5.9 | 5.9 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 |
| 2 | 20 | 20 | 20 | 34.2 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | 20*, 10 | 10 | 30 | 8.9 | 12.0 | -35.4 | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 |
| 4 | 20 | 20 | 20 | 30.2 | 0.0 | 100.0 | 2.4 | 2.4 | 14.5 | 14.5 | 14.5 | 14.5 | 14.5 | 14.5 | 14.5 | 14.5 | 14.5 | 14.5 | 14.6 |
| 5 | 20 | 20 | 20 | 8.1 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 6 | 20 | 20 | 20 | 36.8 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 7 | 20 | 20 | 20 | 29.0 | 7.6 | 74.0 | 31.7 | 37.5 | 37.5 | 37.5 | 37.5 | 37.5 | 37.5 | 37.5 | 37.5 | 37.5 | 37.5 | 37.5 | 37.5 |
| 7 | 20, 20 | 20 | 40 | 34.6 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 3.4 | 27.0 | 27.4 | 27.4 | 27.4 | 27.4 | 27.4 | 27.4 | 27.4 | 27.4 |
| 8 | 20, 10, 10 | 20 | 20 | 14.6 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 23.9 |
| 8 | 20, 10, 10 | 10 | 30 | 7.2 | 17.4 | -141.7 | 21.0 | 22.9 | 23.3 | 23.9 | 23.9 | 23.9 | 23.9 | 23.9 | 23.9 | 23.9 | 23.9 | 23.9 | 23.9 |
| 8 | 20, 10, 10 | 10 | 40 | 22.8 | 8.4 | 63.0 | 17.1 | 18.2 | 18.3 | 19.3 | 19.3 | 19.3 | 19.3 | 19.3 | 19.3 | 19.3 | 19.3 | 19.3 | 19.3 |
| 9 | 20*, 20 | 20 | 40 | 7.2 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 | 7.5 |
| 10 | 20, 10*, 10 | 20 | 20 | 17.5 | 0.0 | 100.0 | 0.0 | 8.9 | 23.9 | 25.4 | 27.4 | 57.9 | 58.7 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 |
| 10 | 20, 10*, 10 | 10 | 40 | 60.0 | 42.2 | 29.6 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 | 60.0 |
| 11 | 10, 10, 20 | 10 | 10 | 26.0 | 8.8 | 66.0 | 11.0 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 |
| 11 | 10, 10, 20 | 10 | 20 | 9.9 | 12.2 | -23.4 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 | 12.2 |
| 11 | 10, 10, 20 | 20 | 40 | 2.5 | 1.5 | 40.3 | 7.0 | 7.8 | 7.8 | 7.8 | 7.8 | 7.8 | 7.8 | 7.8 | 7.8 | 7.8 | 7.8 | 7.8 | 7.8 |
| 12 | 20, 10 | 20 | 20 | 3.4 | 0.0 | 100.0 | 7.7 | 11.0 | 11.0 | 11.0 | 11.0 | 11.0 | 11.0 | 11.0 | 11.0 | 11.1 | 11.1 | 11.1 | 23.3 |
| 12 | 20, 10 | 10 | 30 | 11.1 | 6.7 | 39.9 | 15.6 | 23.3 | 23.3 | 23.3 | 23.3 | 23.3 | 23.3 | 23.3 | 23.3 | 23.3 | 23.3 | 23.3 | 23.3 |
| 13 | 20, 20 | 20 | 20 | 29.4 | 0.0 | 100.0 | 4.7 | 9.1 | 16.7 | 16.7 | 16.7 | 16.7 | 16.7 | 16.7 | 16.7 | 16.7 | 16.7 | 16.7 | 16.7 |
| 13 | 20, 20 | 20 | 40 | 16.7 | 2.3 | 86.4 | 2.3 | 3.4 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 |
| 14 | 20, 20 | 20 | 20 | 47.7 | 14.7 | 69.3 | 19.8 | 25.0 | 27.2 | 29.0 | 31.9 | 36.2 | 36.2 | 36.2 | 36.2 | 36.2 | 36.2 | 36.2 | 36.2 |
| 14 | 20, 20 | 20 | 40 | 35.0 | 20.8 | 40.5 | 25.2 | 25.9 | 26.5 | 26.5 | 26.5 | 26.5 | 26.5 | 26.5 | 26.5 | 26.5 | 26.5 | 26.5 | 26.5 |
| 15 | 20*, 10, 10 | 10 | 30 | 30.6 | 41.3 | -35.2 | 41.3 | 43.2 | 43.2 | 43.2 | 43.2 | 43.2 | 43.2 | 43.2 | 43.2 | 43.2 | 43.2 | 43.2 | 43.2 |
| 15 | 20*, 10, 10 | 10 | 40 | 39.8 | 35.1 | 12.0 | 42.6 | 42.6 | 42.6 | 42.6 | 42.6 | 42.6 | 42.6 | 42.6 | 42.6 | 42.6 | 42.6 | 42.6 | 42.6 |
| 16 | 20, 10 | 20 | 20 | 23.3 | 0.7 | 96.9 | 16.0 | 27.7 | 27.7 | 27.7 | 27.7 | 27.7 | 27.7 | 27.7 | 27.7 | 27.7 | 27.7 | 27.7 | 27.7 |
| 16 | 20, 10 | 10 | 30 | 20.1 | 12.9 | 36.0 | 12.9 | 12.9 | 12.9 | 12.9 | 12.9 | 12.9 | 12.9 | 12.9 | 12.9 | 12.9 | 12.9 | 12.9 | 12.9 |
| 17 | 20*, 20 | 20 | 40 | 20.9 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 6.0 | 6.0 | 6.0 | 6.0 | 7.7 |
| 18 | 20, 20 | 20 | 20 | 19.8 | 0.0 | 100.0 | 15.2 | 15.2 | 22.1 | 22.1 | 22.1 | 22.1 | 22.1 | 27.8 | 27.8 | 27.8 | 27.8 | 27.8 | 31.9 |
| 18 | 20, 20 | 20 | 40 | 21.9 | 15.8 | 27.8 | 18.6 | 20.9 | 31.9 | 31.9 | 31.9 | 31.9 | 31.9 | 31.9 | 31.9 | 31.9 | 31.9 | 31.9 | 31.9 |
| 19 | 20 | 20 | 20 | 1.7 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.9 |

*Phenobarbitone (PB) dose given but not during electrographic seizures. Yellow: first dose is analyzed (n=14). Green: second dose is analyzed (n=13). Blue: third dose is analyzed (n=4). Pink: time between doses calculated.

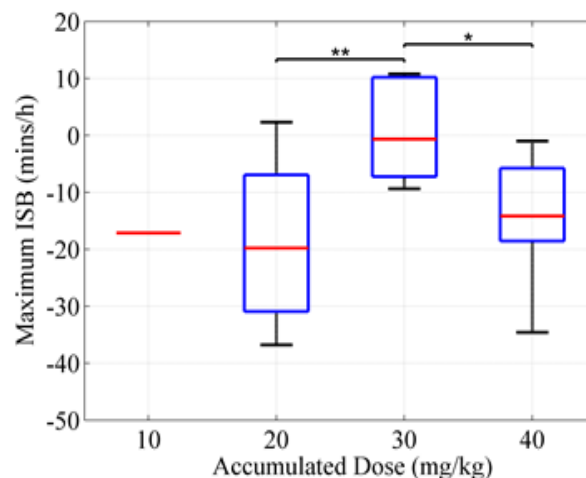
Comparison of T₋₁ vs T₊₁ by dosage (10 and 20 mg/kg)

A subgroup analysis was performed by splitting phenobarbitone into individual dosages. The differences between the maximum ISB pre- and one hour post-phenobarbitone administration depended on dosage ($p=0.002$). Compared with doses of 10 mg/kg, phenobarbitone doses of 20 mg/kg resulted in an immediate and significant reduction in the maximum ISB [mean difference (95% CI) of 20 mg/kg ($n=20$) vs 10 mg/kg ($n=11$)] group: -18.19 (-23.67 to -12.70) vs -4.43(-11.45 to 2.59) minutes/hour].

Comparison of T₋₁ vs T₊₁ by accumulated dosage

One neonate had an accumulated dosage of 10 mg/kg and was excluded from the statistical analysis. The differences between maximum ISB pre- and one hour post-phenobarbitone administration depended on accumulated dosage of 20, 30 or 40 mg/kg ($p=0.006$). The reduction in maximum ISB was greatest for the 20 mg/kg dose [mean difference (95% CI): -19.0 (-25.0 to -12.9) minutes/hour ($n=14$)], followed by the 40 mg/kg dose [mean difference (95% CI): -14.3 (-21.3 to -7.4) minutes/hour ($n=10$)] and the 30 mg/kg dose [mean difference (95% CI): -1.0 (-10.0 to 8.0) minutes/hour ($n=6$)]. Pairwise comparisons revealed statistically significant differences between the 30 mg/kg dose and both the 20 mg/kg and 40 mg/kg dose ($p=0.001$ and 0.017 respectively). No statistically significant differences were found between the 20 mg/kg and the 40 mg/kg dose ($p=0.247$) (figure 8.6).

Figure 8.6 The change in maximum instantaneous seizure burden (ISB) based on dosages
The change in maximum instantaneous seizure burden (ISB) due to the administration of phenobarbitone ($N_{10\text{mg/kg}}=1$ dose, $N_{20\text{mg/kg}}=14$ doses, $N_{30\text{mg/kg}}=6$ doses, $N_{40\text{mg/kg}}=10$ doses). The accumulated doses were estimated assuming maintenance doses of phenobarbitone accounting for clearance. *denotes $p<0.05$ and ** denotes $p<0.01$.



Comparison of seizure burden between effective and ineffective doses (20 mg/kg)

The seizure burden at the time of phenobarbitone administration was significantly lower for effective doses [mean (95% CI): 28.1 (-5.9 to 62.1) minutes/hour (n=13)] than ineffective doses [mean (95% CI): 117.6 (71.3 to 164.0) minutes/hour (n=7)]; $p=0.004$. Ten of the 13 effective doses were the first doses of phenobarbitone.

Analysis on second-line anti-seizure medication

There were 13 doses of second-line anti-seizure medication (phenytoin, midazolam) given to 10 of the 18 neonates with electrographic seizures. Of these, 12 doses were concurrently administered with evidence of electrographic seizures in 9 neonates (6 on phenytoin, 3 on midazolam). The maximum ISB was significantly reduced immediately after the administration of the second-line anti-seizure medication from a median (interquartile ranges) of 9.7 (5.9-27.5) minutes/hour during a 1 hour period before the administration, to a median of 0 (0-5.9) minutes/hour during a 1 hour period after the administration ($p=0.004$).

No seizures were observed during a 1 hour period after the administration of the second-line anti-seizure medication in 5 of 9 neonates. This reduction was also significant in the longer term as the maximum ISB in the remaining hours after the last dose of second-line anti-seizure medication administration, with a median (IQR) of 6.1 (0.0-15.0) minutes/hour; $p=0.008$. In all 14 excluded neonates who received phenobarbitone, seizures returned despite the administration; seven of whom received second-line anti-seizure medication (4 had phenytoin, 3 had midazolam).

8.6 Discussion

Using an innovative method of analysis with time series, we found that phenobarbitone has an immediate effect in reducing electrographic seizure burden in term neonates; this reduction however was only temporary with seizures returning in the majority of neonates after the last dose of phenobarbitone administration. Phenobarbitone as an anti-seizure medication was effective in reducing the seizure burden in term neonates only up 4 hours after the administration.

Timing of phenobarbitone administration

In terms of the timing of administration, the age of phenobarbitone administration are often not specified by authors (van den Broek et al., 2012; van Rooij et al., 2010b). In a cohort of neonates with HIE (≥ 34 weeks), prophylactic phenobarbitone at 20 mg/kg given within 6 hours of life was shown to significantly reduce the incidence of seizures [8% in phenobarbitone group (n=2/25) vs 40% in control group (n=8/20)]; however treatment was based only on clinical seizures (Singh et al., 2005).

Prolonged seizure duration is thought to potentiate the risk of permanent brain damage and increases the difficulty of stopping seizure activity (Ben-Ari, 2006; Holmes and Ben-Ari, 2003; Lado et al., 2002), thus generating the hypothesis that the best chance of terminating a seizure is with early treatment (Abend and Wusthoff, 2012). Since ongoing seizures lasting for at least 30 minutes have been shown to cause neuronal injury in neonatal animal models with ischaemia (Fujikawa, 2005; Klitgaard et al., 2002); indicating that perhaps in clinical practice, treatment should be instigated as early as within 30 minutes from the onset of seizures. In this study, the age of phenobarbitone administration was variable. When there were ongoing electrographic seizures, the median time from onset of electrographic seizure activity to treatment was approximately at 1.9 hours. If treatment was instigated earlier or soon after the onset of electrographic seizures, better response to phenobarbitone treatment may be observed.

This cohort of neonates is not a homogenous population but consisted of differing aetiologies. The severity of injury varies, with different underlying mechanism of pathophysiology, each contributing differently to the seizure pathway. This may lead to different characteristics of how seizures recurred or ended. In determining neonatal outcome from seizures or other brain insults, it is vital to know the pre-existing condition of the fetal or neonatal brain (Gluckman and Williams, 1992). Together with pharmacoresistance, there may be other independent and multifactorial factors fuelling the seizure pathway causing some seizures to abate or continued or with some observed to return to pre-treatment level 4 hours after phenobarbitone administration. It would not be justified to standardize a physiological explanation to each individual neonate studied in this group. GABA may play a role but applying its

direct interpretation from other experimental studies to the findings of this research study beckons caution.

Exposure of phenobarbitone to neonatal brain in the absence of seizures has been shown to stunt the development of both excitatory and inhibitory synaptic function, indicating functional neurotoxicity. Even brief treatment with phenobarbitone, carbamazepine, diazepam or valproate have been shown to cause an increase apoptotic neural death in normal immature rodents, leading to the stagnation in growth and development of the developing brain (Bittigau et al., 2002). In the critical phases of microgenesis and during the process of neuronal pruning, apoptosis in the neurones which causes cell death to take place is known to be a normal component of brain development during the first 10 days of life (Bittigau et al., 2002). Therefore, careful interpretation of drug-induced apoptosis cannot be taken conclusively in the presence of the normal processes of brain development which involves apoptosis.

Timing of anti-seizure medication administration

One limitation of this study is that the first dose of phenobarbitone was variable and that this was at the discretion of the attending neonatologist. Clinical scenarios such as apnoeic seizures and concerns in an already sedated neonate may lead neonatologists to administer the lower first dose of phenobarbitone to avoid further respiration depression. The dose and timing of anti-seizure medication and their effects on electrographic seizures in human term neonates have not been well studied. At what appropriate dose of anti-seizure medication and how soon and of what frequency should neonatal seizures be treated?

Most experts recommend early cessation of the use of anti-seizure medication due to concerns over their side effects, combined with the fact that neonatal seizures typically abate within days with no intervention and have a low risk of early recurrence (Guillet and Kwon, 2007),. However, it remains unethical for clinicians not to treat neonatal seizures. In most clinical settings, phenobarbitone currently remains the first-line anti-seizure medication in most neonatal units to treat neonatal seizures (Bartha et al., 2007), despite being shown to be effective in approximately 50% of cases (Booth and Evans, 2004; Rennie and Boylan, 2003).

In this study, the reduction of electrographic seizures burden was less significant with time after its administration, and that second-line anti-seizure medication are usually needed. During the initial stage of treatment, the response of seizures to first-line anti-seizure medication is unpredictable, prompting the use of second-line anti-seizure medication in most cases if there were persistence of clinical seizures. However, there is variation as to the choice of the second-line anti-seizure medication. This limitation is in accordance with the literature that there is still no consensus among neonatologists today as to what incremental dose of phenobarbitone and which type of second or third-line anti-seizure medication should be appropriate when first-line anti-seizure medication such as phenobarbitone failed to control the seizures (Bartha et al., 2007; Boylan et al., 2002) (further discussion previously in Section 2.4: Using anti-seizure medication to treat neonatal seizures).

Treatment of neonatal seizures needs to reflect effectiveness and perhaps we need to change our current strategy of treating neonatal seizures in our neonatal units by using 20 mg/kg up to a total dose of 40 mg/kg, and that 10 mg/kg after a 20 mg/kg dose may be ineffective. Perhaps the second loading dose should be given within 4 hours after the first loading dose in order for phenobarbitone to reduce seizure burden effectively.

Analysis of seizure burden

The analysis undertaken for this study is novel because it assessed seizure burden by taking into consideration the total seizure burden per hour continuously during the entire EEG monitoring in order to generate a time series to evaluate the instantaneous seizure burden in a continuous hourly fashion before and after the administration of phenobarbitone. This method of analysis gives more accuracy in delineating the response of electrographic seizures to phenobarbitone. Future studies should include robust methods of analyses to assess the effectiveness of treatment with anti-seizure medication based on the response of electrographic seizures rather than clinical seizures.

However, methods of analysing electrographic seizures can only be made available when there is ongoing multichannel EEG monitoring. Furthermore, electroclinical dissociation of seizures have been shown to increase after treatment with anti-

seizure medication, indicating the need for continuous multichannel EEG monitoring during seizure treatment in neonates (Boylan et al., 2013; Clancy, 2006a) as up to 80% of neonatal seizures are not detectable by the human eye (Scher et al., 2003). Apart from causing the electroclinical dissociation of seizures, phenobarbitone has also been shown to reduce the EEG amplitude (Mathieson SR et al., 2014).

When a seizure event is suspected, an EEG monitoring is usually warranted to confirm these seizures. Since studies have shown that seizures can be harmful to the developing neonatal brain, it is crucial to treat these seizures appropriately with the most effective anti-seizure medication timely and with consistency if better neurodevelopmental outcome is to be expected. However, due to lack of evidence in effectiveness, some authors have decided that the use of anti-seizure medication in the immediate period following perinatal asphyxia cannot be recommended for routine clinical practice and is only justifiable to be using anti-seizure medication for prolonged and frequent clinical seizures (Booth and Evans, 2004).

8.7 Conclusions

Phenobarbitone as an anti-seizure medication was effective in reducing seizure burden in term neonates only up 4 hours after the administration; therefore neonatologists should be aware that seizures can recur and return with greater intensity particularly only after the first dose of phenobarbitone. Most of what we understand about the paradoxical effect of GABA on the developing brain is derived from animal studies and may have merit, but the situation in the human neonate might be very different and more complex.

We have clearly shown an immediate reduction in seizures in all neonates following a dose of 20 mg/kg of phenobarbitone. Doses of phenobarbitone at 20 mg/kg as subsequent dose after the initial loading dose of 20 mg/kg, rather than 10 mg/kg as subsequent doses were significantly more effective in reducing seizure burden. In the majority of neonates the effect is not sustained and seizures returned. The mechanism of phenobarbitone action is clearly complex and it may be that its effect on seizure reduction may not be, at least initially, mediated by the GABA_A receptor; further research should delve into investigating the precise mechanism of how phenobarbitone works in the human developing neonatal brain.

Perhaps our current treatment strategy to treat neonatal seizures with anti-seizure medication needs to be evaluated and that the multichannel EEG monitoring is warranted to ensure whether there are ongoing seizures since most neonatal seizures are subclinical in nature. Future studies should focus on assessing the effectiveness of anti-seizure medication on electrographic seizures using the multichannel EEG accompanied with a robust method of analysis of seizure burden if the clinical incentive is to abolish seizures efficiently in the human developing neonatal brain.

What this study adds?

- Phenobarbitone reduces electrographic seizures temporarily in the majority of neonates for approximately 4 hours.
- Loading doses at 20 mg/kg rather than 10 mg/kg were more effective in reducing seizure burden.
- Phenobarbitone may be more effective if treatment strategies are tightly aligned with EEG monitoring.

Chapter 9

The Dissociation of Electroclinical Seizures in Term Neonates

9.1 Abstract

Background: An estimate of the incidence of electroclinical dissociation (ECD) of seizures may be a useful tool to neonatologists in the management of neonatal seizures in term neonates.

Purpose: To determine the rate the electroclinical dissociation of seizures in term neonates with varying aetiologies.

Methods: Electrographic seizures were annotated by an experienced neonatal electroencephalographer. Simultaneous video was reviewed in each neonate and the ECD index was defined as the accumulated number of electrographic-only seizure relative to the total number of seizures in each neonate according to the respective diagnoses. Data are expressed as medians (interquartile ranges).

Results: Twenty-four neonates with electrographic seizures had simultaneous video-EEG monitoring. There were 8 cooled neonates with hypoxic-ischaemic encephalopathy (HIE), 4 non-cooled HIE and 12 neonates with other diagnoses [stroke (n=6), benign seizures (n=2), intraparenchymal haemorrhage (n=2), subdural haemorrhage (n=1) and seizure of unknown cause (n=1)]. EEG monitoring began at age 9 (5-28) hours, EEG duration was 69 (49-104) hours and age of first EEG seizure was 19 (11-36) hours.

Of the 24 neonates, 19 had electrographic seizures and 5 had electroclinical seizures only. There was no significant difference in the ECD index between the cooled neonates with HIE and all the non-cooled neonates with HIE ($p=0.109$), with neonates with stroke ($p=0.465$) and those other diagnoses identified ($p=0.893$). Although there was no statistical significance between the groups, the ECD index in the cooled neonates with HIE, in non-cooled neonates with HIE, neonates with focal stroke and in neonates with other diagnoses were 88 (55-100)%, 94% (small number $n=3$), 64 (58-68)% and 75 (61-89)% respectively.

Conclusion: Based on our current cohort, the occurrence of ECD is high. This emphasizes the need for continuously multichannel video EEG monitoring as the majority of electrographic seizures is not detected by clinical observation in the NICU.

9.2 Introduction

The incidence of electroclinical dissociation (ECD) of seizures in neonates has been reported to be as high as 80 % of neonates treated with anti-seizure medication (Boylan et al., 1999; Boylan et al., 2002; Castro, Jr. et al., 2005; Scher et al., 2003); particularly implicating phenobarbitone (Boylan et al., 2002). Phenobarbitone may have facilitated the occurrence of ECD and different mechanisms in the developing human neonatal brain are responsible for the clinical and EEG manifestation of seizures at the molecular level (Boylan et al., 1999). With the advent of therapeutic hypothermia, ECD has also been reported in neonates (Nash et al., 2011; Wusthoff et al., 2011; Yap et al., 2009). The severity of EEG background activity, status epilepticus and higher seizure burden have also been implicated in the increasing occurrence of ECD (Boylan et al., 1999; Pinto and Giliberti, 2001).

As most seizures in neonates are subclinical, continuous multichannel video-EEG is crucial in monitoring ECD in neonates, particularly during the treatment period. Neonates with ECD have been shown to have higher seizure burden and have poorer neurodevelopmental outcome (Weiner et al., 1991). Most studies have reported only the number of neonates affected by ECD (Nash et al., 2011; Wusthoff et al., 2011; Yap et al., 2009); apart from cooling, these studies did not make any other direct associations with other factors such as the usage of anti-seizure medication, the degree of severity of brain injury, status epilepticus and seizure burden.

What is already known on this topic?

- Electroclinical dissociation of seizures can occur up to 80% of neonates treated with anti-seizure medication.
- The electroclinical dissociation index in term neonates in the current era of care remains unknown.

9.3 Aims

Hypothesis: There is a high incidence of electroclinical dissociation (ECD) of seizures in term neonates. A new and current cohort of neonates with seizures including cooled neonates is needed to confirm and quantify this, so as to determine the dissociation rate of seizures according to different seizure aetiologies.

Study aim: To determine the characteristics of electroclinical dissociation (ECD) of seizures in term neonates (the Electroclinical dissociation study). Electroclinical dissociation of seizures is believed to be a common phenomenon, but has rarely been quantified using multichannel EEG and in a cohort of term neonates who were either cooled or non-cooled with multiple aetiologies. This study aimed to determine the occurrence of this phenomenon in the current population of term neonates with seizures.

9.4 Methods

The methodology of this study is described in detail in Chapter 5 (Methodology).

The electroclinical dissociation of seizure (ECD) index was used for this study to ascertain the current status of ECD in a cohort of term neonates with simultaneously multichannel video-EEG monitoring. The ECD index of seizure was defined as the percentage of the accumulated number of electrographic-only seizures relative to the total number of seizures in each neonate. The ECD index was determined between neonates who had received phenobarbitone at 20 mg/kg vs those who had received phenobarbitone at 40 mg/kg, between neonates who had higher seizure burden (defined as those who had total seizure burden of >60 mins) vs those who had lower seizure burden (defined as those who had total seizure burden of <60 mins), and between neonates with status epilepticus vs those who did not have status epilepticus.

Statistical analysis: Data were expressed as medians and interquartile ranges (IQR). All statistical analyses were performed using SPSS Statistics 20.0 (IBM SPSS Statistics, Illinois, USA). All tests were two-sided; p value <0.05 was considered to be statistically significant.

9.5 Results

During the study period from 2009 to 2011, twenty-four neonates in CUMH with seizures and simultaneous long-term video-EEG monitoring were identified (figure 9.1). The median (interquartile ranges) age when EEG monitoring began was 9.95 (3.3-18.7) hours, EEG duration was 67.5 (46.7-91.27) hours and age of first EEG seizure was 18.99 (13-38.89) hours. The aetiologies for seizures included hypoxic-ischaemic encephalopathy (HIE) (n=12), stroke (n=6), benign seizures (n=2), intraparenchymal haemorrhage (n=2), subdural haemorrhage (n=1) and seizure of unknown cause (n=1). Table 9.1 lists the clinical demographics and table 9.2 lists the details of EEG monitoring and anti-seizure medication given in 12 neonates with HIE (4 of whom were non-cooled) and 12 neonates with other diagnoses (all non-cooled).

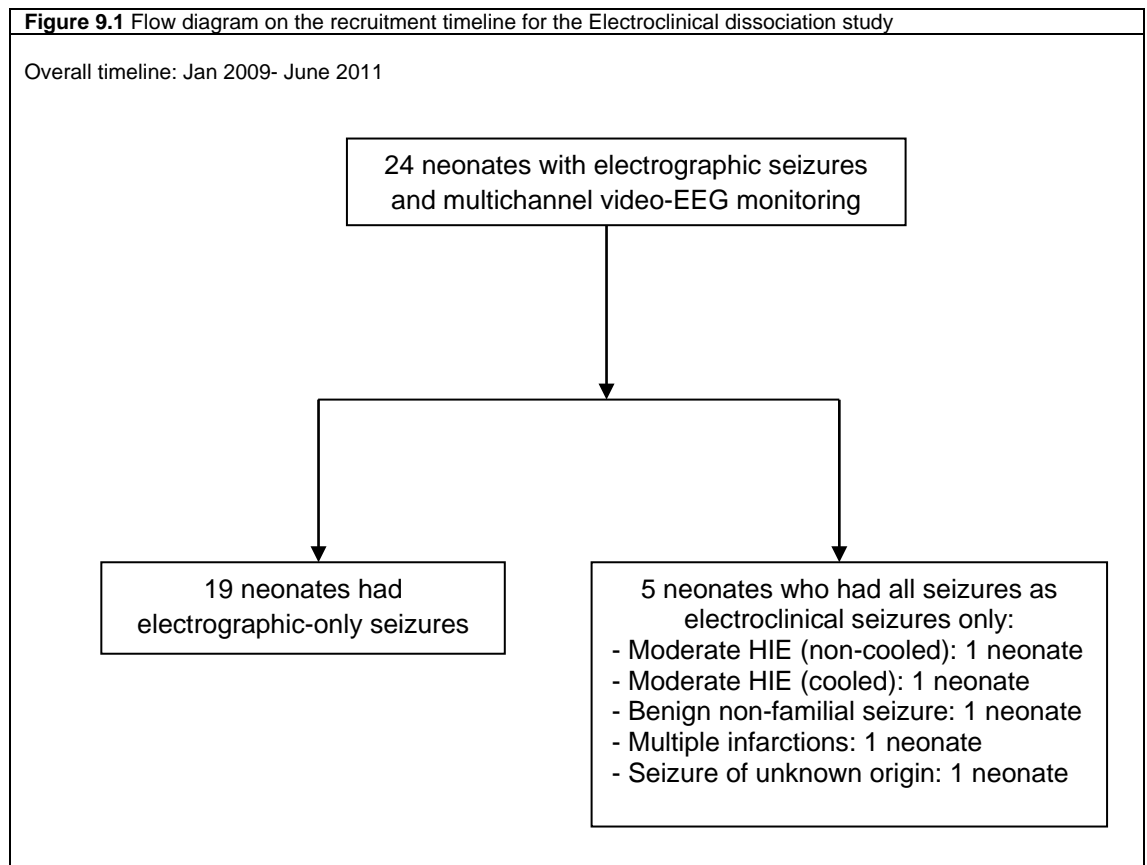


Table 9.1 Individual clinical characteristics of 12 neonates with electrographic seizures due to HIE and 12 neonates with electrographic seizures arising from non-HIE conditions in the order of increasing seizure burden

| Case | 12 neonates with HIE (grade) | Cooling duration (age in hours) | GA (weeks) | BW (grams) | Sex | Sentinel before delivery | Mode of delivery | 5 min Apgar score | First pH |
|---|--|---------------------------------|------------|------------|-----|---|--------------------|-------------------|----------|
| 1 | 3 | 72 (0.25-72.3) | 41 | 4130 | F | Deep decelerations in the second stage, post-dates, FTP | IOL | 0 | 7.052 |
| 2 ^E | 2 | Non-cooled | 41 | 3570 | M | Early decelerations, post-dates, FTP | IOL-Ventouse | 6 | 6.97 |
| 3 | 2 | 72 (2.5-74.5) | 40 | 3940 | F | Fetal bradycardia, antenatal pelvicalyceal dilatation | Ventouse | 3 | 7.047 |
| 4 | 2 | Non-cooled | 40 | 3000 | F | None | SVD | 3 | 6.99 |
| 5 ^E | 2 | 72 (2-74) | 40 | 4290 | 1 | Shoulder dystocia | SVD | 3 | 6.93 |
| 6 | 3 | 72 (2-74) | 40 | 3880 | F | Head high, difficult delivery, meconium-stained liquor | EMCS | 5 | 6.81 |
| 7 | 2 | 72 (3-75) | 40 | 3000 | F | Post-dates | IOL-Forceps | 4 | 7.1 |
| 8 ^E | 2 | 72 (2-74) | 40 | 3140 | F | Severe antepartum haemorrhage, fetal bradycardia | EMCS | 4 | 6.626 |
| 9 ^E | 3 | 65 (0.8-66) | 41 | 5190 | 1 | Shoulder dystocia (23mins delivery between head and body) | SVD | 0 | 7 |
| 10 ^E | 2 | Non-cooled | 39 | 2950 | 1 | Failed ventouse | Forceps | 10 | 7.396 |
| 11 ^R | 3 | 72 (6-78) | 37 | 2900 | 1 | Decreased fetal movement, non-reassuring cardiotocogram | ELCS before labour | 6 | 7.18 |
| 12 ^E | 3 | Non-cooled | 40 | 3350 | 1 | Prolonged second stage, spinal pain, failed ventouse, meconium-stained liquor | IOL-Forceps | 7 | 7.34 |
| 12 neonates with other diagnoses | | | | | | | | | |
| 13 | Benign familial neonatal seizures | | 39 | 3300 | M | Fetal bradycardia at second stage | Ventouse | 9 | 7.17 |
| 14 | Benign non-familial seizures | | 41 | 4200 | M | None | SVD | 9 | 7.323 |
| 15 | Subdural haemorrhage | | 40 | 3710 | F | None | SVD | 10 | 7.394 |
| 16 | Multiple infarctions | | 39 | 2930 | F | None | SVD | 9 | 7.36 |
| 17 | Multiple infarctions | | 40 | 3520 | F | Maternal pre-eclampsia toxemia, FTP at second stage | IOL-Forceps | 10 | not done |
| 18 | Unknown | | 39 | 3740 | M | Low lying placenta, polyhydramion, PROM 36h | IOL | 9 | 7.04 |
| 19 | Arterial ischaemic stroke-LMCA | | 41 | 3400 | F | None | SVD | 10 | 7.385 |
| 20 | Non-traumatic intraparenchymal haemorrhage | | 41 | 3360 | M | Persistent occiput-posterior presentation | IOL-Forceps | 10 | 7.349 |
| 21 ^E | Traumatic intraparenchymal haemorrhage | | 41 | 3420 | F | Post-dates | IOL-Ventouse | 7 | 7.34 |
| 22 ^E | Arterial ischaemic stroke-LMCA | | 39 | 3160 | M | Non-reassuring cardiotocogram, prolonged labour (25h) | EMCS | 10 | 7.30 |
| 23 ^E | Arterial ischaemic stroke-LMCA | | 38 | 3370 | M | Previous section | ELCS | 10 | 7.38 |
| 24 ^E | Arterial ischaemic stroke-LMCA and LPCA | | 39 | 3300 | M | Fetal bradycardia at second stage | Ventouse | 9 | 7.17 |

BW: birthweight; C: neonates from Cork University Maternity Hospital; E: neonates with status epilepticus; ELCS: elective C-section; FTP: failure to progress; GA: gestational age; IOL: induction of labour; L: neonates from University College London Hospital; LMCA: left middle cerebral artery; LPCA: left posterior cerebral artery; R: neonates with EEG seizures following discontinuation of cooling; RMCA: right middle cerebral artery; SVD: spontaneous vaginal delivery.

Table 9.2 Details on the sequence of anti-seizure medication given and reasons why ongoing EEG seizures were not treated

| Case | Age of first clinical seizure (hours) | Characteristic of first clinical seizure | Age of EEG monitoring (hours) | Age of first EEG seizure (hours) | Duration of EEG monitoring (hours) | Age of first anti-seizure medication (hours) | Order of anti-seizure medication given during EEG (mg/kg) | Reasons for not given further anti-seizure medication despite ongoing EEG seizures |
|-----------------|---------------------------------------|---|-------------------------------|----------------------------------|------------------------------------|--|---|--|
| 1 | 5 | Myoclonic jerks with desaturations | 1.6 | 13 | 92.28 | 6.2 | PB (20) | Saturday 0200-0900. Sedated with no further clinical signs noted |
| 2 ^E | 6 | Limbs jerking and blinking | 2.9 | 7 | 60.92 | 7.3 | PB (20): non-cooled | Complete resolution of seizures |
| 3 | 11 | Hypertonic limbs with arching | 4.7 | 11 | 78.9 | 11 | PB (20, 10) | Saturday 0200-0300. Sedated with no further clinical signs noted |
| 4 | Not recorded | Fisting and posturing | 12.1 | 22 | 97.08 | None | None: non-cooled | Saturday 0030-0500, Sunday 2300, Monday 0012 |
| 5 ^E | 2 | Upper limbs jerks | 4 | 8 | 77.12 | 9.9 | PB (20) | Sunday 0649-0759. Complete resolution of seizures |
| 6 | 1 | Upper limbs jerks, nystagmus to the right | 9.1 | 19 | 159.65 | 1.1 | PB (20, 10, 10), PT, CL, LV | Given for clinical seizures |
| 7 | 4 | Tremulous right hand | 3.3 | 23 | 84.25 | 7.3 | PB (20) | Monday 0036-0241, 0500-1600. Sedated with no further clinical signs noted |
| 8 ^E | 1 | Generalize tonic clonic after intubation | 2.7 | 13 | 101.25 | 15.6 | PB (20) | Wednesday 1500-1700, 2100 to Thursday 0900. Sedated with no further clinical signs noted |
| 9 ^E | 2 | Right arm jerking | 2.8 | 17 | 88.23 | 17.1 | PB (20, 20), PT | Sedated with no further clinical signs noted |
| 10 ^E | 2 | Right sided limbs jerking | 10.8 | 11 | 49.42 | 10.2 | PB (20): non-cooled | Friday 0500-1430 |
| 11 ^R | 2 | Desaturations while on ventilator | 4.8 | 43 | 122.15 | 56.6 | PB (10,10,20), PT,CL | Sunday 0200 (Valentine's day) to Monday 1000. Sedated with no further clinical signs noted |
| 12 ^E | 1 | Eyes staring | 16.8 | 17 | 110.73 | 18.2 | PB (20, 20): non-cooled | Saturday 2300, Sunday 0200, 0500, 2200 |
| 13 | Day 7 | Limbs jerking | 151.3 | 153 | 33.45 | 150.4 | PB (20): non-cooled | Tuesday 0007-0400; only 2 mins long seizure |
| 14 | Day 4 | Upper limb hypertonic, left eye deviation | 119.4 | 121 | 42.63 | 123 | PB (20), PY: non-cooled | Tuesday 1600-1700 |
| 15 | Not recorded | Desaturations | 6.8 | 7 | 56.53 | 8.6 | PB (20, 10, 10), PT, CL, LV, PY: non-cooled | Saturday 0900-1700 |
| 16 | Day 5 | Left focal seizures | 113 | 113 | 14.87 | 115.2 | PB(20): non-cooled | Friday 1800-2030. Complete resolution of seizures |
| 17 | 13 | Left focal seizures | 17.2 | 28 | 45.07 | 18.8 | PB(20, 20): non-cooled | Tuesday 0000,0100,0200, Wednesday 0830 |
| 18 | 42 | Limbs jerking | 33.9 | 34 | 64.83 | 30.5 | PB (20, 20), PT: non-cooled | Saturday 0730-1700 |
| 19 | 54 | Desaturations only | 59.2 | 60 | 69 | 55.8 | PB(20, 10),PT: non-cooled | Saturday 1530-2200 |
| 20 | 18 | Right focal seizures | 21.1 | 22 | 61.02 | 19.6 | PB(20): non-cooled | Saturday 0000-1200. Sedated with no further clinical signs noted |
| 21 ^E | 8 | Left focal seizures | 17.7 | 18 | 73.27 | 10.3 | PB (20, 10), PT: non-cooled | Thursday 1700 to Wednesday 0600 |
| 22 ^E | 33 | Right sided jerks | 3.3 | 39 | 45.63 | 34.4 | PB(20, 10), PT: non-cooled | Thursday 0600-1700 |
| 23 ^E | 15 | Right upper limb jerks | 17.9 | 19 | 51.82 | 23.7 | PB(20, 20): non-cooled | Friday 0230-0730, 1030-1130, 1400-2330. Saturday 0230-1400 |
| 24 ^E | 18 | Right upper limb jerks | 18.9 | 19 | 62.68 | 19 | PB(20, 10, 10), PT: non-cooled | Thursday 1700-0000. Friday 0000 to Saturday 0400 |

B: neonates who had all phenobarbitone doses given before EEG monitoring commence; N: neonates with no anti-seizure medication given at any stage; *neonates who were given at least one of the phenobarbitone dose during EEG monitoring, but all phenobarbitone doses were not given during EEG seizures.

ECD index

The ECD index in the cooled neonates with HIE (n=7), in non-cooled neonates with HIE (n=3: 2 moderate HIE, 1 severe HIE), neonates with focal stroke (n=4) and in neonates with other diagnoses (n=5) were 88 (55-100)%, 94% (small number n=3), 64 (58-68)% and 75 (61-89)% respectively.

Neonates with no occurrence of ECD

Of 24 neonates with electrographic seizures, five neonates (cases 2, 5, 14, 16 and 18) did not have any ECD of seizures (i.e. which were detected as electrographic-only seizures) as all their seizures were presented as electroclinical seizures only (see table 9.3). Case 2 was a non-cooled neonate with moderate HIE, who had one period of status epilepticus lasting 37 minutes (all were electroclinical seizures which consisted of jerking limb movements and only received one dose of phenobarbitone which essentially led to the termination of electrographic seizures).

Case 5 was a cooled neonate with moderate HIE who presented with upper limb jerking movements and was given one dose of phenobarbitone with subsequent complete resolution of seizures. Case 5 had 2 electroclinical seizures (1 clonic seizures, 1 subtle seizure (staring episode); with a total seizures burden of 58 minutes). Case 14 was a non-cooled neonate who presented with stiffening of the upper limbs and eye deviation; phenobarbitone and pyridoxine were administered before a diagnosis of exclusion was made (the subsequent diagnosis was benign non-familial seizures). Case 14 had 4 electroclinical seizures (3 clonic seizures, 1 subtle seizure which presented as mouthing episode) and 1 seizure event which was obscured by video-imaging; total seizure burden was 4 minutes.

Case 16 was a non-cooled neonate who presented with left focal seizures and was noted to have multiple infarctions on the MRI; one dose of phenobarbitone was given which essentially led to a termination of EEG seizures. This baby had 4 seizures (3 clonic seizures, 1 seizure obscured by video-imaging) with a seizure burden lasting 12 minutes. Case 18 was a non-cooled neonate who presented with jerking movements of the limbs; phenobarbitone and phenytoin were given before the diagnosis of seizures of unknown origin was made. This baby had all 8 electroclinical seizures presented as upper limb clonic movements clinically (with a seizure burden

lasting 25 minutes. However, most clinical seizures in Case 18 were somehow not detected and not treated.

In the remaining 19 neonates, 1123 seizures were analyzed. One neonate (case 1) had 44% of seizures which were identified as electrographic-only seizures (EOS) while the remaining 18 neonates had more than >50% of their total number of seizures detected as EOS [median (IQR)=80.03 (54.73-95.01)%]. Three (cases 4, 7, 9) of the 18 neonates had all of their seizures identified as EOS. Case 4 was a non-cooled neonate with moderate HIE with no anti-seizure medication given. Case 4 had 7 seizures with a seizure burden lasting 51 minutes. Case 7 was a cooled neonate who had moderate HIE and had received phenobarbitone 20 mg/kg. Case 7 had 54 seizures with a seizure burden lasting 78 minutes. Case 9 was a cooled neonate with severe HIE and had received total phenobarbitone dose of 40 mg/kg. Case 9 had 49 seizures with a seizure burden lasting 198 minutes.

Table 9.3 Characteristics of EEG seizures

| Case | Seizure burden (mins) | Number of seizures (n) | Mean seizure duration (seconds) | Seizure window (hours) | EEG-only seizures (n) | Clonic seizures (n) | Subtle seizures (n) | Obscured seizures (n) | Electroclinical seizures (n) | EEG-only seizures (%) | Conic seizures (%) | Subtle seizures (%) | Obscured seizures (%) | Electroclinical seizures (%) |
|------|-----------------------|------------------------|---------------------------------|------------------------|-----------------------|---------------------|------------------------------|-----------------------|------------------------------|-----------------------|--------------------|---------------------|-----------------------|------------------------------|
| 1 | 24 | 9 | 161 | 9 | 4 | 0 | 5 (B) | 0 | 5 | 44.44 | 0 | 55.56 | 0 | 55.56 |
| 2 | 37 | 1 | 2207 | 1 | 0 | 1 (L-sided) | 0 | 0 | 1 | 0 | 100 | 0 | 0 | 100 |
| 3 | 44 | 21 | 127 | 11 | 17 | 1 (RUL) | 3 (B, T) | 0 | 4 | 80.95 | 4.76 | 14.29 | 0 | 19.05 |
| 4 | 51 | 7 | 438 | 29 | 7 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 |
| 5 | 58 | 2 | 1741 | 1 | 0 | 1 (arching) | 1 (St) | 0 | 2 | 0 | 50 | 50 | 0 | 100 |
| 6 | 67 | 89 | 45 | 118 | 49 | 1 (UL) | 35 (B, Bx, Cy, St) | 4 | 36 | 55.06 | 1.12 | 39.33 | 4.49 | 40.45 |
| 7 | 78 | 54 | 87 | 15 | 54 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 |
| 8 | 119 | 18 | 396 | 17 | 16 | 0 | 1 (Hi) | 1 | 1 | 88.89 | 0 | 5.56 | 5.56 | 5.56 |
| 9 | 198 | 49 | 243 | 41 | 49 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 | 0 |
| 10 | 213 | 70 | 182 | 31 | 66 | 1 (RUL) | 2 (Cr) | 1 | 3 | 94.29 | 1.43 | 2.86 | 1.43 | 4.29 |
| 11 | 225 | 198 | 68 | 51 | 183 | 0 | 1 (D) | 14 | 1 | 92.42 | 0 | 0.51 | 7.07 | 0.51 |
| 12 | 637 | 271 | 141 | 92 | 217 | 20 (arching, UL) | 24 (B, D, Hb, Hy, M, Sh, St) | 10 | 44 | 80.07 | 7.38 | 8.86 | 3.69 | 16.24 |
| 13 | 2 | 4 | 28 | 3 | 3 | 1 (LLL) | 0 | 0 | 1 | 75 | 25 | 0 | 0 | 25 |
| 14 | 4 | 5 | 43 | 19 | 0 | 3 (L-sided) | 1 (M) | 1 | 4 | 0 | 60 | 20 | 20 | 80 |
| 15 | 7 | 5 | 81 | 8 | 4 | 0 | 0 | 1 | 0 | 80 | 0 | 0 | 20 | 0 |
| 16 | 12 | 4 | 183 | 2 | 0 | 3 (LUL) | 0 | 1 | 3 | 0 | 75 | 0 | 25 | 75 |
| 17 | 18 | 6 | 176 | 8 | 4 | 0 | 2 (Cy) | 0 | 2 | 66.67 | 0 | 33.33 | 0 | 33.33 |
| 18 | 25 | 8 | 185 | 9 | 0 | 8 (RUL) | 0 | 0 | 8 | 0 | 100 | 0 | 0 | 100 |
| 19 | 67 | 41 | 98 | 9 | 27 | 0 | 10 (D) | 4 | 10 | 65.85 | 0 | 24.39 | 9.76 | 24.39 |
| 20 | 98 | 36 | 163 | 12 | 35 | 1 (LLL) | 0 | 0 | 1 | 97.22 | 2.78 | 0 | 0 | 2.78 |
| 21 | 142 | 9 | 944 | 28 | 5 | 3 (LUL) | 1 (Cl) | 0 | 4 | 55.56 | 55.56 | 11.11 | 0 | 44.44 |
| 22 | 201 | 23 | 523 | 11 | 13 | 0 | 9 (M, Su) | 1 | 9 | 56.52 | 56.52 | 39.13 | 4.35 | 39.13 |
| 23 | 266 | 112 | 143 | 21 | 77 | 17 (RUL) | 15 (M, Su) | 3 | 32 | 68.75 | 68.75 | 13.39 | 2.68 | 28.57 |
| 24 | 327 | 101 | 195 | 35 | 62 | 16 (RUL) | 19 (Cy, M, Su, Y) | 4 | 35 | 61.39 | 61.39 | 18.81 | 3.96 | 34.65 |

B: blinking; Bx: boxing; Cl: clenching of fists; Cr: crying; Cy: cycling of limbs; D: desaturations of peripheral oxygen; Hb: head bobbing; Hi: hiccups; Hy: hyperventilating; L: left; LLL: left lower limb; M: mouthing; RUL: right upper limb; Sh: shivering; St: staring; Su: sucking; T: twitching of left upper limb; UL: upper limb; Y: yawning.

Cooling vs non-cooled neonates

Based on 19 neonates with electrographic-only seizures, there was no significant difference between the cooled (n=7) vs all non-cooled neonates (n=12) in terms of the percentage of the number of seizures ($p=0.498$) identified. There was no significant difference between cooled (n=7) vs non-cooled neonates with HIE (n=3) in terms of the percentage of the number of seizures ($p=0.564$).

If comparisons were made between cooled HIE (n=7) vs non-cooled neonates with focal arterial ischaemic stroke (n=4), there is also no difference in the percentage of the number of seizures identified ($p=0.465$) (table 9.4). There was no significant difference in the dissociation of electroclinical seizures between cooled (n=7) vs non-cooled neonates with HIE [albeit the number of cases is small (n=7 vs 3) ($p=0.109$) and when comparison was made between cooled vs all non-cooled neonates with various diagnoses (excluding stroke) (n=7 vs 5) ($p=0.893$).

Table 9.4 Comparison of ECD of seizures between cooled and non-cooled neonates

| Case | Diagnoses | EEG-only seizures (n) of the total number of seizures (n) | EEG-only seizures (%) |
|--------------------------|--|---|-----------------------|
| Cooled (n=7) | | | |
| 1 | HIE 3 | 4 of 9 | 44.44 |
| 7 | HIE 2 | 54 of 54 | 100.00 |
| 8 | HIE 2 | 16 of 18 | 88.89 |
| 3 | HIE 2 | 17 of 21 | 80.95 |
| 6 | HIE 3 | 49 of 89 | 55.06 |
| 11 | HIE 3 | 183 of 198 | 92.42 |
| 9 | HIE 3 | 49 of 49 | 100.00 |
| Non-cooled (n=12) | | | |
| 4 | HIE 2 | 7 of 7 | 100.00 |
| 10 | HIE 2 | 66 of 70 | 94.29 |
| 12 | HIE 3 | 217 of 271 | 80.07 |
| 20 | Non-traumatic intraparenchymal haemorrhage | 35 of 36 | 97.22 |
| 21 | Traumatic intraparenchymal haemorrhage | 5 of 9 | 55.56 |
| 19 | Arterial ischaemic stroke-LMCA | 27 of 41 | 65.85 |
| 22 | Arterial ischaemic stroke-LMCA | 13 of 23 | 56.52 |
| 17 | Multiple infarctions | 4 of 6 | 66.67 |
| 13 | Benign familial neonatal seizures | 3 of 4 | 75.00 |
| 15 | Subdural haemorrhage | 4 of 5 | 80.00 |
| 24 | Arterial ischaemic stroke-LMCA and LPCA | 62 of 101 | 61.39 |
| 23 | Arterial ischaemic stroke-LMCA | 77 of 112 | 68.75 |

Phenobarbitone dosage

All but 1 (case 4) of the 24 neonates who received phenobarbitone (10, 3 and 10 neonates were given accumulative dose of phenobarbitone at 20, 30 and 40 mg/kg respectively). Based on 19 neonates, between those who had received phenobarbitone 20 mg/kg (n=6) vs phenobarbitone 40 mg/kg (n=9), there was no significant difference found in terms of the percentage of the number of seizures (p=0.516) identified (table 9.5).

Table 9.5 Comparison of ECD of seizures in neonates based on total phenobarbitone dosages

| Case | EEG-only seizures (n) of the total number of seizures (n) | EEG-only seizures (%) |
|--|---|-----------------------|
| No phenobarbitone given | | |
| 4 | 7 of 7 | 100 |
| Total phenobarbitone dosage at 20 mg/kg (n=6) | | |
| 1 | 4 of 9 | 44.44 |
| 7 | 54 of 54 | 100 |
| 8 | 16 of 18 | 88.89 |
| 20 | 35 of 36 | 97.22 |
| 21 | 5 of 9 | 55.56 |
| 10 | 66 of 70 | 94.29 |
| Total phenobarbitone dosage at 30 mg/kg (n=3) | | |
| 19 | 27 of 41 | 65.85 |
| 22 | 13 of 23 | 56.52 |
| 3 | 17 of 21 | 80.95 |
| Total phenobarbitone dosage at 40 mg/kg (n=9) | | |
| 6 | 49 of 89 | 55.06 |
| 11 | 183 of 198 | 92.42 |
| 9 | 49 of 49 | 100 |
| 17 | 4 of 6 | 66.67 |
| 13 | 3 of 4 | 75 |
| 15 | 4 of 5 | 80 |
| 24 | 62 of 101 | 61.39 |
| 23 | 77 of 112 | 68.75 |
| 12 | 217 of 271 | 80.07 |

Severity of brain injury

Based on 19 neonates who had electrographic-only seizures, there was no significant difference in the percentage of the number of seizures between neonates who had severe HIE (n=5) vs those who had moderate HIE (n=5); $p=0.169$. Also, there was no significant difference between those who had severe HIE (n=5) vs all other diagnoses (n=14) in terms of the percentage of the number of seizures ($p=0.711$) (table 9.6).

Table 9.6 Comparison of ECD of seizures between cooled and non-cooled neonates

| Case | Diagnoses | EEG-only seizures (n) of the total number of seizures (n) | EEG-only seizures (%) |
|------------------------------|--|---|-----------------------|
| HIE (n=10) | | | |
| 1 | Severe | 4 of 9 | 44.44 |
| 6 | Severe | 49 of 89 | 55.06 |
| 11 | Severe | 183 of 198 | 92.42 |
| 9 | Severe | 49 of 49 | 100 |
| 12 | Severe | 217 of 271 | 80.07 |
| 7 | Moderate | 54 of 54 | 100 |
| 8 | Moderate | 16 of 18 | 88.89 |
| 3 | Moderate | 17 of 21 | 80.95 |
| 4 | Moderate | 7 of 7 | 100 |
| 10 | Moderate | 66 of 70 | 94.29 |
| Other diagnoses (n=9) | | | |
| 20 | Non-traumatic intraparenchymal haemorrhage | 35 of 36 | 97.22 |
| 21 | Traumatic intraparenchymal haemorrhage | 5 of 21 | 55.56 |
| 19 | Arterial ischaemic stroke-LMCA | 27 of 41 | 65.85 |
| 22 | Arterial ischaemic stroke-LMCA | 13 of 23 | 56.52 |
| 17 | Multiple infarctions | 4 of 6 | 66.67 |
| 13 | Benign familial neonatal seizures | 3 of 4 | 75 |
| 15 | Subdural haemorrhage | 4 of 5 | 80 |
| 24 | Arterial ischaemic stroke-LMCA and LPCA | 62 of 101 | 61.39 |
| 23 | Arterial ischaemic stroke-LMCA | 77 of 112 | 68.75 |

Status epilepticus

Based on 19 neonates, the number of electrographic-only seizures between neonates who had status epilepticus (n=8) vs those who had no status epilepticus (n=11) was not significant, in terms of the percentage of the number of seizures ($p=0.804$) (table 9.7).

Table 9.7 Comparison of ECD of seizures between neonates with and without status epilepticus

| | EEG-only seizures (n) of the total number of seizures (n) | EEG-only seizures (%) |
|---|--|-----------------------|
| Status epilepticus cases (n=8) | | |
| 8 | 16 of 18 | 88.89 |
| 9 | 49 of 49 | 100 |
| 10 | 66 of 70 | 94.29 |
| 21 | 5 of 9 | 55.56 |
| 22 | 13 of 23 | 56.52 |
| 23 | 77 of 112 | 68.75 |
| 24 | 62 of 101 | 61.39 |
| 12 | 217 of 271 | 80.07 |
| No status epilepticus cases (n=11) | | |
| 1 | 4 of 9 | 44.44 |
| 7 | 54 of 54 | 100 |
| 3 | 17 of 21 | 80.95 |
| 6 | 49 of 89 | 55.06 |
| 11 | 183 of 198 | 92.42 |
| 4 | 7 of 7 | 100 |
| 20 | 35 of 36 | 97.22 |
| 19 | 27 of 41 | 65.85 |
| 17 | 4 of 6 | 66.67 |
| 13 | 3 of 4 | 75 |
| 15 | 4 of 5 | 80 |

Seizure burden

Based on 19 neonates who had electrographic-only seizures at higher seizure burden (>60 mins) (n=13) vs lower seizure burden (<60 mins) (n=6), the proportion in terms of the percentage of the number of seizures was not significant (p=0.792), but there was a significant difference in terms of the number of seizures (p=0.002) identified in neonates who had higher seizure burden (table 9.8).

Table 9.8 Comparison of ECD of seizures between neonates with higher versus lower seizure burden

| | Seizure burden (minutes) | EEG-only seizures (n) of the total number of seizures (n) | EEG-only seizures (%) |
|--|-----------------------------|--|-----------------------|
| Cases with higher seizure burden (n=13) | | | |
| 6 | 67 | 49 of 89 | 55.06 |
| 7 | 78 | 54 of 54 | 100 |
| 8 | 119 | 16 of 18 | 88.89 |
| 9 | 198 | 49 of 49 | 100 |
| 10 | 213 | 66 of 70 | 94.29 |
| 11 | 225 | 183 of 198 | 92.42 |
| 12 | 637 | 217 of 271 | 80.07 |
| 19 | 67 | 27 of 41 | 65.85 |
| 20 | 98 | 35 of 36 | 97.22 |
| 21 | 142 | 5 of 9 | 55.56 |
| 22 | 201 | 13 of 23 | 56.52 |
| 23 | 266 | 77 of 112 | 68.75 |
| 24 | 327 | 62 of 101 | 61.39 |
| Cases with lower seizure burden (n=6) | | | |
| 1 | 24 | 4 of 9 | 44.44 |
| 3 | 44 | 17 of 21 | 80.95 |
| 4 | 51 | 7 of 7 | 100 |
| 13 | 2 | 3 of 4 | 75 |
| 15 | 7 | 4 of 5 | 80 |
| 17 | 18 | 4 of 6 | 66.67 |

9.6 Discussion

Based on findings of multichannel video-EEG, this study has shown that the index of electroclinical dissociation of seizures remains high in term neonates who were treated with anti-seizure medication. The ECD index in the cooled neonates with HIE, in non-cooled neonates with HIE, neonates with focal stroke and in neonates with other diagnoses were approximately 88%, 94%, 64% and 75% respectively. This study has demonstrated that ECD is high in HIE and other seizure aetiologies. Clinical surveillance will not accurately measure response to anti-seizure medication in neonates.

Few theories have been hypothesized on the mechanism pertaining to the electroclinical dissociation (ECD) of seizures in term neonates at the molecular level, and this has been discussed in section 3.1.2. Regional interconnectivity, including interhemispheric as well as corticospinal, which are not fully mature due to incomplete myelination of white matter tracts have been implicated in leading to only modest or no behavioral manifestations of these seizures.

Neonates can show no signs or very subtle tonic or clonic movements, often limited to only one limb, making the diagnosis difficult to discern from myoclonus or other automatisms (Boylan et al., 2013; Mizrahi EM and Kellaway P, 1998). The sedative effect of phenobarbitone may account for this, as it is also known to be a potent benzodiazepine. In some cases in this study, there were no clinical signs detected after neonates had at least one dose of phenobarbitone administered, however the number of neonates in this study is small and a larger cohort of neonates is required to confirm this finding.

Electroclinical dissociation of seizures and therapeutic hypothermia

Electroclinical dissociation of seizures was also common in neonates treated with therapeutic hypothermia (Nash et al., 2011; Wusthoff et al., 2011; Yap et al., 2009) (previously discussed in section 6.6 of Chapter 6: the Cooling study). Yap *et al.* monitored a cohort of 20 neonates (13 moderate HIE, 7 severe HIE) with selective head cooling (Yap et al., 2009). The occurrence of electrographic-only seizures in this study by Yap *et al.* is higher; the study mainly used the aEEG and single-channel EEG tracing, with intermittent multichannel EEG for seizure detection they may have underestimated the true seizure burden.

Using the gold standard: multichannel video-EEG from 26 neonates who received whole-body cooling, (reviewed by 2 pediatric neurophysiologists), Wusthoff *et al.* detected 17/26 (65%) neonates who had EEG seizures during and immediately after cooling; 8/17 (47%) had only electrographic-only seizures [13/17(76%) with seizure onset within 48 hours of life]. The onset of seizures spanned from 6 to 95 hours of age; this study advocated that EEG monitoring should be extended beyond 24 hours for neonates receiving therapeutic hypothermia (Wusthoff et al., 2011).

When comparing results from this study with studies conducted by Wusthoff *et al.* and Yap *et al.*, there was no significant difference in the dissociation of electroclinical seizures between cooled (n=7) vs non-cooled neonates with HIE [albeit the number of cases is small (n=7 vs 3) and when comparison was made between cooled vs all non-cooled neonates with various diagnoses (n=7 vs 12).

Using continuous video-EEG (commenced at 10.2 ± 2.9 hours of age for 90.9 ± 28.3 hours) and whole body cooling in 41 neonates treated with anti-seizure medication (lorazepam, phenobarbitone, fosphenytoin, levetiracetam), Nash *et al.* detected electrographic seizures in 14/41 (34%) [13/14 had seizure onset within 18 hours of age, 8 neonates within 6 hours of age] (Nash *et al.*, 2011). Perhaps the differing treatment strategies in other institution with the use of a different first-line anti-seizure medication (lorazepam) may have explained the lower incidence of electrographic-only seizures in these studies when compared to the results of this research study.

Electroclinical dissociation of seizures and anti-seizure medication

In 16 (cases 1, 2, 3, 5, 7, 8, 9, 11, 12, 14, 15, 16, 17, 22, 23, 24) of the 24 neonates in this study group, EEG monitoring commenced before the first anti-seizure medication was administered (table 9.2). The limitation of this study is that the numbers are small to make the comparison of ECD of seizures before and after treatment; it would be interesting to assess this in a larger cohort.

The incidence of ECD has been thought to be high in neonates treated with anti-seizure medication (Boylan *et al.*, 1999; Boylan *et al.*, 2002; Boylan *et al.*, 2013; Castro, Jr. *et al.*, 2005; Scher *et al.*, 2003); implicating specifically phenobarbitone which remains the most common first-line anti-seizure medication in most neonatal unit worldwide. In a cohort of 88% of neonates who were treated with anti-seizure medication, up to 79% of neonates had EEG seizures with no clinical correlates (Clancy and Legido, 1987).

ECD was noted in 58% of neonates with electroclinical seizures after the first-line (phenobarbitone) or second-line (phenytoin) anti-seizure medication (Scher *et al.*, 2003). In a study by Scher *et al.*, uncoupling was defined as persistence of EEG seizure despite suppression of $\geq 50\%$ of clinical seizures after 1 or 2 anti-seizure medication (this was based on a cohort of neonates with gestational ranging from

25 to 43 weeks with continuous 24 hour EEG monitoring performed) (Scher et al., 2003).

In study of Boylan *et al.* and Painter *et al.*, phenobarbitone and phenytoin were used and may have facilitated the occurrence of ECD (Boylan et al., 2002; Painter et al., 1999). However, anti-seizure medication were administered in 49% of ECD seizures and 68% of electroclinical seizures, suggesting that anti-seizure medication was not the only factor in causing seizures to dissociate (Weiner et al., 1991). This raises the possibility that other mechanisms in the developing neonatal brain, may be responsible for the clinical and EEG manifestation of seizures (Scher et al., 2003).

Severity of brain lesions and background EEG

In a group of neonates who had electroclinical dissociation of seizures, 83% of neonates had more severe background EEG compared to 69% of neonates with electroclinical seizures. The association between ECD and with severe EEG background has been implicated in 6 neonates (gestational ages ranging from 25 to 41 weeks) monitored from 100 to 360 minutes by Boylan *et al.*, (Boylan et al., 1999) and in 11 neonates in a study by Pinto *et al.* (Pinto and Giliberti, 2001). In 11 of 30 term neonates with HIE, electroclinical dissociation of seizures was constantly identified only in neonates with depressed and undifferentiated background EEG (defined as EEG activity between 5 and 15 μ V) which is indicative of severe cerebral injury. This implies that electroclinical dissociation of seizures is more common in neonates with severe HIE (Boylan et al., 1999).

Status epilepticus

Electroclinical dissociation of seizures caused by the progression of status epilepticus has been a hypothesis considered (Watanabe, 2014). Electroclinical dissociation has been noted as a feature of prolonged status epilepticus in adults and children (Abend et al., 2013b; Abend et al., 2013a; Watanabe, 2014); as prolonged status epilepticus does cause an adult or older children to be progressively in a state of severe encephalopathy, or when status occurs in the presence of a severe underlying encephalopathy.

In neonates without pre-existing brain damage, frequent seizures *per se* may cause mild depression in the EEG background activity characterized by the loss of high voltage slow patterns, an important constituent of slow wave sleep reflecting cortico-cortical connectivity. Mild depression only in the acute stage is not associated with neurological sequelae, but previously damaged brain may be more vulnerable than normal brain (Abend et al., 2013b; Watanabe, 2014).

In rodent studies using kainate induced status epilepticus, the higher dose of kainate (15 mg/kg) emulating a more severe brain injury than a lower dose (5 mg/kg) causes more frequent occurrence of electroclinical dissociation of seizures (Mikati et al., 2003). They tended to occur when there was continuous ictal activity with flat tracing ($<10 \mu\text{V}$) and when there were epileptiform spike and sharp waves repeating at a rate of once every 2 seconds.

In a neonatal study which included 17 neonates (31 to 41 weeks gestation) with a mean (range) EEG recording of 97 (60-181) minutes, there was a significant correlation between the tendency towards status epilepticus and the occurrence of electroclinical dissociation of seizures (Biagioni et al., 1998). This event has been hypothesized to be due to the impairment of transmission between the motor cortex and the lower structures of the brain and muscle (Wasterlain et al., 2010). This leads to the progression of EEG changes and a diminution of clinical expression as status epilepticus progress (Boylan et al., 2013; Treiman, 1995).

Neonatal studies have reported a small incidence of status epilepticus in cohorts of neonates who were cooled. In 8 /17 (47%) neonates who had electrographic-only seizures, 4/17 (23%) had status epilepticus (Wusthoff et al., 2011), while 3/6 with electrographic-only seizures had status epilepticus (Nash et al., 2011)]. In our study, there were more number of seizures per neonate in the ECD (n=6) than in the electroclinical group (n=4); $p<0.05$.

Monitoring of electroclinical dissociation of seizures in neonates

As the application of multichannel video-EEG can be difficult to obtain on an emergent basis in many neonatal intensive care units, amplitude integrated EEG (aEEG) devices are generally utilized (Azzopardi, 2015; Boylan et al., 2010; Boylan et al., 2013; Boylan and Pressler, 2013; Gupta et al., 2015). The interpretation on the aEEG is based on a trend display, which shows a heavily

time-compressed signal after it has been extensively filtered (as described in chapter 3). In the aEEG, seizures are detected by acute alterations in spectral width, and a raw EEG from both the single and 2-channels can be accessed by the viewer for confirmation. Several reports now indicate that the aEEG has relatively high specificity but compromised sensitivity, detecting approximately 75% of seizures detected from conventional full lead montage EEG (Shellhaas et al., 2007; Tekgul et al., 2005).

In neonates with electroclinical dissociation of seizures and those with electroclinical only seizures, there was no significant demographic difference found in a study conducted by Weiner *et al.* (Weiner et al., 1991). Sixteen percent of neonates demonstrated ECD; this is however an underestimation of seizure burden because they did not have continuous video-EEG monitoring performed (Weiner et al., 1991). Some studies have reported a high occurrence of electroclinical dissociation of seizures in the neonatal population (as high as 80%) (Scher et al., 2003); early, prolonged and continuous multichannel video-EEG monitoring is essential for confirmation of this phenomenon and for assessing effectiveness of treatment with anti-seizure medication (Azzopardi, 2015; Boylan et al., 2010; Boylan et al., 2015; Pressler et al., 2015; Shellhaas, 2015). Also, since multifocal seizures can occur in neonates, the multichannel video-EEG is the more sensitive than the aEEG in detecting these types of seizures.

Most electrographic seizures emerged during out-of hours working time (past midnight and during weekends); there were no alarm systems to alert both the nursing and medical personnel when there were ongoing electrographic seizures detected on the multichannel video-EEG monitoring device. Hence, treatment of seizures remains suboptimal as many neonates were treated when there were abnormal movements with no electrographic seizure correlates. Further trials on assessing the effectiveness of treatment would be highly optimized using the automated seizure detection embedded in the EEG system on a continuous monitoring basis, as a method to alert neonatologists to treat when there are ongoing electrographic seizures and not to treat when there are no electrographic seizures.

9.7 Conclusions

The high incidence of ECD raised the important issue of accurate seizure detection if our goal is to optimize neuroprotection in neonates. The findings from this study are important, making it crucial that we develop a more effective method of detecting seizures. Further research should revisit our inevitable reliance on the continuous and prolonged multichannel video-EEG monitoring for seizure surveillance in neonates.

What this study adds?

- Using the multichannel video-EEG, this study has reported the occurrence of electroclinical dissociation of seizures in our current population of term neonates in the NICU.
- Although the cohort of this study is small, the index of electroclinical dissociation of seizures is found to be high in term neonates treated: the ECD index in the cooled neonates with HIE, in non-cooled neonates with HIE, neonates with focal stroke and in neonates with other diagnoses were 88%, 94%, 64% and 75% respectively.

Section 4

Summary

Chapter 10

Summary, Clinical implications and Implications for Future Research

I began this thesis as a clinical neonatologist with a special interest in neonatal seizures. I soon realized how very little I knew about the subject. Neither did I realise that I was on the cusp of a wave of real breakthroughs in the understanding of the newborn brain, how it develops, how seizures develop, and how these seizures may be harmful. I began to see, through careful analysis of hundreds of EEGs with the help of my mentors, how many seizures were missed by clinicians and other non-seizures, misread and mistreated as seizures. I found myself immersed into a research group that was desperately aware of these problems, including the inadequacy of current pharmacologic interventions entrusted as treatment for neonatal seizures. I also found myself closely involved in the one new therapy for hypoxic-ischaemic encephalopathy that showed real promise of improved neonatal neurodevelopmental outcome following hypoxic-ischaemic encephalopathy: therapeutic hypothermia.

After a long, uncertain and sometimes exhausting journey, I can now summarize, with the help of many dedicated and sagacious colleagues, that this thesis contributes the following novel contributions to the literature of neonatal seizures. We have:

- Presented in-depth information on the characteristics of seizures based on current population of neonates in the NICU through early and prolonged continuous EEG recording during this current era of neonatal care.
- Demonstrated, using the multichannel EEG, that the recorded electrographic seizure burden is decreased in neonates with hypoxic-ischaemic encephalopathy who were cooled, when compared with neonates who were non-cooled. This was the first study which used early, prolonged and continuous multichannel EEG to quantify the seizure burden between non-cooled and cooled term neonates with hypoxic-ischaemic encephalopathy.
- Postulated that therapeutic hypothermia may possess some anti-seizure properties, since it has the ability to reduce the electrographic seizure burden in term neonates who were cooled.

- Shown that electrographic seizures in neonates with stroke have a particular focal sharp wave/spike-polyspike pattern and phase reversal is frequently present. Using the multichannel video-EEG, this was the first study to report that in neonates with stroke, the background EEG shows asymmetry and suppression over the infarcted side; characteristic unilateral bursts of theta activity, sharp waves and spikes were present.
- Shown that approximately 80% of the total seizure burden in term neonates with stroke is not recognized clinically without the use of continuous multichannel video-EEG monitoring.
- Demonstrated that phenobarbitone reduces seizure burden only on a temporary basis in most neonates and that 20 mg/kg dose may be more effective than 10mg/kg dose.
- Shown that phenobarbitone may be more effective if treatment strategies are tightly aligned with EEG monitoring.
- Presented an electroclinical dissociation of seizure index (ECD index) for hypoxic-ischaemic encephalopathy (ECD index=90%) and seizures due to other diagnoses including stroke (ECD index=60%) and shown that the occurrence of the dissociation of electroclinical seizures remains high in our current population of neonates in the NICU.
- Created a large, unique bio-bank of neonatal seizures in term neonates with multiple aetiological origins, with which will play a key role in in-depth research on neonatal seizure.

Implications of this research for clinical practice

During the course of this thesis, I worked in 3 different neonatal intensive care units as part of my clinical rotations. Clinical guidelines on the treatment of neonatal seizures in these hospitals were similar and each hospital had adopted therapeutic hypothermia as their standard of care for neonates with hypoxic-ischaemic encephalopathy. The results from this research study (the Phenobarbitone study) has shown that in a group of term neonates with differing aetiologies, using phenobarbitone at 20 mg/kg was more effective than using 10 mg/kg as the first loading dose to control seizures more effectively, and that better seizure control may be achieved if the second 20 mg/kg of phenobarbitone (up to a total dose of 40 mg/kg) was administered within 4 hours after its first administration.

Neonates with seizures who were treated with phenobarbitone ultimately could be treated more effectively if treatment was anchored and controlled under tight EEG monitoring and that electrographic seizures were treated early. Early and appropriate treatment relies on early identification of these seizures on the multichannel EEG. However, early, prolonged and continuous monitoring with multichannel EEG alone is insufficient when dealing with a neonate with seizures either stemming from HIE, stroke or when treatment with phenobarbitone has been instigated. Findings from this research study have shown that the phenomenon of electroclinical dissociation (the Electroclinical Dissociation study) of seizures is still very prevalent in our current cohort of term neonates; they will escape detection without early, prolonged and continuous multichannel EEG monitoring.

The characteristics of electrographic seizures and seizure burden in term neonates who had stroke (the Stroke study) provided invaluable information for clinical management among neonatologists in terms of early diagnosis and treatment. The findings from this research study have shown that in the absence of therapeutic hypothermia as a treatment option, seizure burden was higher than expected in this group of neonates with stroke who were treated with phenobarbitone (Low et al., 2014). Perhaps better treatment strategies focusing on treating seizures stemming from neonatal stroke are required. In this current era of neonatal care, therapeutic hypothermia has not as yet been recommended for stroke; it is still being investigated (Harbert et al., 2011; van der Worp et al., 2010) and there is no firm conclusion as yet but cooling may emerge as one of the potential treatment in the near future for perinatal stroke.

During this study, with the help of my colleagues, we had collected EEG from at least 214 neonates and analyzed up to approximately 6089 seizures in total. Based on early, prolonged and continuous multichannel EEG monitoring, the findings from this research study have provided new information on seizure burden based on a heterogeneous population of term neonates which reflects the current environment and the 'real-world' data remain crucial to clinicians in managing neonates with seizures in most NICUs today. The findings from this research study (the Cooling study) has shown that the seizure burden in term neonates with hypoxic-ischaemic encephalopathy is reduced by therapeutic hypothermia, when compared with those who were not treated with this method in a historical cohort (Low et al., 2012a).

For neonatologists, this research study highlights that many seizures were missed; despite ongoing monitoring with the prolonged multichannel video-EEG, we are still not good in detecting seizures. As a result some neonates were treated who did not need to be and other neonates who needed treatment were missed. As a result of my research for this thesis, I am convinced that neonatal seizures are harmful and they need to be treated and we need more effective anti-seizure medications. Continuous interpretation of the multichannel EEG is urgently required as it plays a pivotal role in alerting the neonatal personnel as part of the clinical management of the neonate.

Decisions have to be made by neurophysiologists in terms of EEG interpretation and by neonatologists in terms of whether to instigate treatment with anti-seizure medication or not. In practice, these decisions depend on various details gathered from history, physical examination and other investigations of the baby with suspected seizures. These findings in combination with a reliable source of interpretation of the multichannel EEG by neurophysiologists in detecting seizures on a 24 hour basis, will enhance our clinical management of dealing with neonates with seizures. Whether they improve outcome remains to be seen. However, we cannot answer this question without accurate seizure burden detection and optimal seizure control.

There are limitations in human raters in interpreting the multichannel EEG because it requires particular skills, which in turn is related to the level of experience of expert interpreters. The automated version of the multichannel EEG [which is the neonatal automated seizure detection algorithm (NASDA)] for interpretation in real-time will enhance further the interpretation by neurophysiologists and the decision-making in clinical management by neonatologists.

Further studies are needed in the development of alert systems, such as a voice alarm or a colour alarm system for automated seizure detection, alerting neonatal personnel in the NICU to seizures detected by the NASDA. Many multi-disciplinary groups around the world are working hard on completing this task (Boylan and Rennie, 2006; Cherian et al., 2011; Stevenson et al., 2013; Temko et al., 2011). Automated detection of seizures such as the NASDA is needed, to aid neonatologists in the instigation of treatment at the appropriate time, in order to curb potential additional damage caused by untreated seizures to the developing neonatal brain.

Implications for future Research

A more in-depth understanding of the pathophysiology of seizures in human neonates is required in order for newer treatment of neonatal seizures to be effective at the cotside. A more strategically approach to the antiseizure medication administration in terms of dosing and timing is required for further analysis of seizure burden in the neonates. Some of the upcoming and new anti-seizure medication such as melatonin (Turgut et al., 2006), topiramate, levetiracetam (Beaulieu, 2013; Loiacono et al., 2014), allopurionol (Chaudhari and McGuire, 2008), xenon (Azzopardi et al., 2013; Dingley et al., 2014; Lobo et al., 2013), stem-cell therapy (Scharfman and McCloskey, 2009) studied on neonates and their effects of what happen to seizures when commenced during therapeutic hypothermia will need to be further assessed (Boylan et al., 2015; Cilio and Ferriero, 2010; Pressler and Mangum, 2013).

Long-term neurodevelopmental outcome studies on neonates with seizures will also be required, as it remains a controversy whether the aetiology, the seizures generated by the aetiology themselves or the side effects of treatment with anti-seizure medication are harmful to the developing neonatal brain. To determine the correlation of simultaneous and multiple factors and effects from various aetiologies, other medications and anti-seizure medications used to treat neonatal seizures, and the resultant variable degrees of adverse long-term neurodevelopmental outcome will add to our understanding, particularly when further attempts are made to improve our clinical management of the neonate in terms of diagnosis, treatment and to provide prognosis when counselling parents in the NICU.

Prolonged and continuous multichannel EEG monitoring remains the gold standard for detecting seizures in neonates (Boylan et al., 2010; Boylan et al., 2002; Clancy, 2006a). Although it is regarded as the most accurate method for confirming neonatal seizures in NICUs worldwide, the limited availability of expert interpreters serves as a major deterrent for routine NICU use. Embedding a neonatal automated seizure detection algorithm (NASDA) into a continuous multichannel EEG system is promising, as it could have the ability to provide continuous and robust interpretation of the multichannel EEG for neonatologists at the cotside in the NICU.

The findings of this research study and the development of the seizure bio-bank have implications leading towards the further in-depth research on neonatal seizures. In addition, an in-depth investigation of the electroclinical dissociation of seizures in neonates with different aetiologies was conducted since video and EEG records were available in neonates monitored. Future studies will provide us more understanding of neonatal seizures may include:

1. A larger sample size of more neonates with HIE and stroke is required to confirm and strengthen the results from this research study.
2. Larger cohort of neonates with well-defined quantitative analysis tool for seizure burden based on more reliable method of seizure detection is required.
3. Since the aEEG is commonly used in most NICUs today, a complete comparison between the aEEG and the multichannel EEG in detecting seizures would be useful information to neonatologists and EEG manufacturers.
4. More translational research needs to be conducted aiming at better understanding of the underlying pathological mechanisms of what cause seizures in the developing neonatal brain so that methods of neuroprotective can effectively treat these seizures. Further understanding of these mechanisms may lead to novel therapies that minimize the chances of adverse outcome which has already instilled by the initial brain injury and which may improve outcome even if injury has occurred.
5. Emerging neuroimaging tool which can study in real-time the neurophysiology of the normal and abnormal human neonatal brain development is required to advance our understanding of neonates at risk of developing seizures.
6. More prospective randomized control trials are required to provide more convincing efficacy of these neuroprotective measures.
7. Better treatment options to treat neonatal seizures apart from phenobarbitone and/ or therapeutic hypothermia should be developed.
8. Seizure detection algorithms, which are limited to the analysis of seizures only, are insufficient to detect 100% of neonatal seizures accurately. Additional physiological markers such as heart rate, respiratory rate, peripheral oxygenation, regional cerebral oxygenation using the near

infrared spectrometry and mean arterial blood pressure are some of the other potential modalities, can be incorporated into the NASDA, and may increase the accuracy of seizures detection. Motion trackers such as those obtained from simultaneous video-EEG recording are also a possibility.

Conclusion

Since neonatal seizures are usually subclinical and are a potential risk factor for poor neurodevelopmental outcome, a continuous automatic online seizure detection system is needed. For neonatologists, a better mode of seizure detection is an important tool to aid neonatologists for short or long term monitoring for the recognition of seizures for diagnosis, and in order to instigate appropriate investigations, to monitor treatment and to counsel parents about prognosis. For researchers, although it seems that monitoring tools and treatment options are limited, further improvement could be achieved.

For the EEG system developers, further tests are ongoing to determine the common sources of false alarms which will help improve and optimize the performance of the NASDA. On the ongoing collaboration between clinical neuroscientists, neurophysiologists, neonatologists, neurologists, neuro-radiologists, pharmacologists, biochemists, biomedical engineers, electrical and electronic engineers, computer programmers, information technologists, statisticians and data analysts will undoubtedly contribute to further optimising seizure detection algorithms in detecting neonatal seizures, with better neuroprotection treatment strategy in mind. The aim to enhance seizure detection in neonates and optimizing seizure treatment in order to improve the long-term neurodevelopmental outcome in neonates continues to motivate research among these multidisciplinary teams which will ultimately bring benefits to hospital policy-makers and health service providers.

Section 5

Contribution of this Thesis to the Literature

Neonatal seizures in hypoxic-ischaemic encephalopathy: the impact of therapeutic hypothermia

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Moderate to severe neonatal hypoxic-ischaemic encephalopathy (HIE) affects approximately 1 to 3 per 1,000 live term births and is a major cause of death and long-term neurodisability (Marlow and Budge, 2005; Lawn *et al.*, 2010). Seizures are the hallmark of neurological injury and approximately 45 to 50% of all neonatal seizures are attributable to HIE (Volpe, 2008). However, neonatal seizures continue to present a diagnostic and therapeutic challenge to clinicians worldwide due to their variable clinical expression and poor response to commonly used antiepileptic drugs (AEDs). The adoption of more widespread EEG monitoring in the neonatal intensive care unit over the last 10 years has meant that the true seizure burden of neonates with HIE has been recognized. Previous research has been hampered by the lack of continuous EEG monitoring to characterize and quantify neonatal seizures in this population. In addition, many studies included seizures with varying aetiologies and EEG monitoring was not continuous in the acute phase of injury.

The evidence of benefit for therapeutic hypothermia in HIE is considered sufficient for the widespread implementation of its use in neonatal intensive care units worldwide (NICE guideline 2010; Jacobs *et al.*, 2013; Harris *et al.*, 2014). A meta-analysis of 3 trials which enrolled 767 neonates showed that therapeutic hypothermia reduced the combined rate of death or disability at 18 months (Edwards *et al.*, 2010). More recently, neonates who were treated with therapeutic hypothermia after perinatal asphyxia have shown improved neurocognitive function at 6 to 7 years of age (Azzopardi *et al.*, 2014). Further data are required to clarify whether therapeutic hypothermia is appropriate for severe HIE. Efforts to supplement therapeutic hypothermia with other neuroprotective agents and to extend the neuroprotection window beyond 72 hours may prove useful for this population (Fan and Van Bel, 2010; Faulkner *et al.*, 2011; Aly *et al.*, 2012; Robertson *et al.*, 2013; Herrera EA *et al.*, 2014; Charriaut-Marlangue *et al.*, 2014).

■ Hypoxic-ischaemic encephalopathy (HIE)

Given that HIE is an evolving process reflecting the evolution of the underlying brain injury (Gunn and Thoresen, 2006), continuous EEG monitoring is essential for assessing cerebral function and for accurate quantification of neonatal seizures. Immediately following the primary hypoxia-ischaemic insult, there is disruption to cerebral oxidative metabolism, cytotoxic oedema develops, excitotoxins accumulate and the EEG is suppressed. Some metabolic recovery is possible over the subsequent 30 to 60 minutes (Tan *et al.*, 1996; Bennet *et al.*, 2007a). A latent phase then follows from about 1 to 6 hours characterized by cerebral hypoperfusion, reduced metabolism and a suppressed EEG. During this period, high energy phosphates return to near normal values (Robertson *et al.*, 2013). However, a secondary injury phase then develops and corresponds to further periods of cytotoxic oedema, accumulation of excitotoxins and hyperperfusion. During this injury phase, there is a failure of cerebral mitochondrial activity (Lorek *et al.*, 1994; Bennet *et al.*, 2006; Wassink *et al.*, 2014) eventually leading to cell death. In moderate to severe brain injury, the background EEG may start to recover during this period and seizures often develop (Figure 1). In very severe injury, the EEG can remain suppressed for days and seizures may never emerge.

■ Seizures and HIE

Seizures are seen in term neonates with significant HIE, usually occurring within the first 24 hours of life (Lynch *et al.*, 2012). In experimental models of HIE, seizures occur either immediately after injury following an asphyxial insult or in a delayed manner 6 to 12 hours after the initial insult when secondary energy failure leads to additional cell death (Scher *et al.*, 2008). Gunn *et al.* found that if ischaemia lasted 30 minutes or longer, a stereotypic sequence of depressed EEG activity followed by a low frequency epileptiform activity was always observed (Gunn *et al.*, 1992). The combination of hypoxia and seizures produces more profound injury in the brain than either factor alone (Wirrell *et al.*, 2001). Seizures add to the hypoxia-ischaemic injury in neonatal animals; the same may be true for neonates (Wirrell *et al.*, 2001; Miller *et al.*, 2002).

In an elegant study by Bjorkman *et al.* using histology, magnetic resonance imaging and spectroscopy, electrographic seizures in piglets were associated with increased severity of brain injury following an extensive hypoxic-ischaemic insult (Bjorkman *et al.*, 2010).

A recent histological study in the hippocampi of 16 deceased full-term asphyxiated neonates has shown that there were more significant increases in microglial activation and expression of the inflammatory markers, namely interleukin 1 β and complement 1q in cases with seizures compared to those without seizures (Schiering *et al.*, 2014). In this study seizures were confirmed with EEG monitoring.

Ideally, accurate identification and quantification of neonatal seizures require continuous multichannel video-EEG monitoring (Boylan and Pressler, 2013). However, continuous video-EEG monitoring is not widely available and as a result, many centres worldwide now use limited two channel aEEG/EEG systems. As long as the limitations of these devices are appreciated, they are still far better than estimating seizure burden using clinical acumen alone (Rennie *et al.*, 2004; Shellhaas *et al.*, 2007; Murray *et al.*, 2008; Malone *et al.*, 2009; Glass *et al.*, 2013).

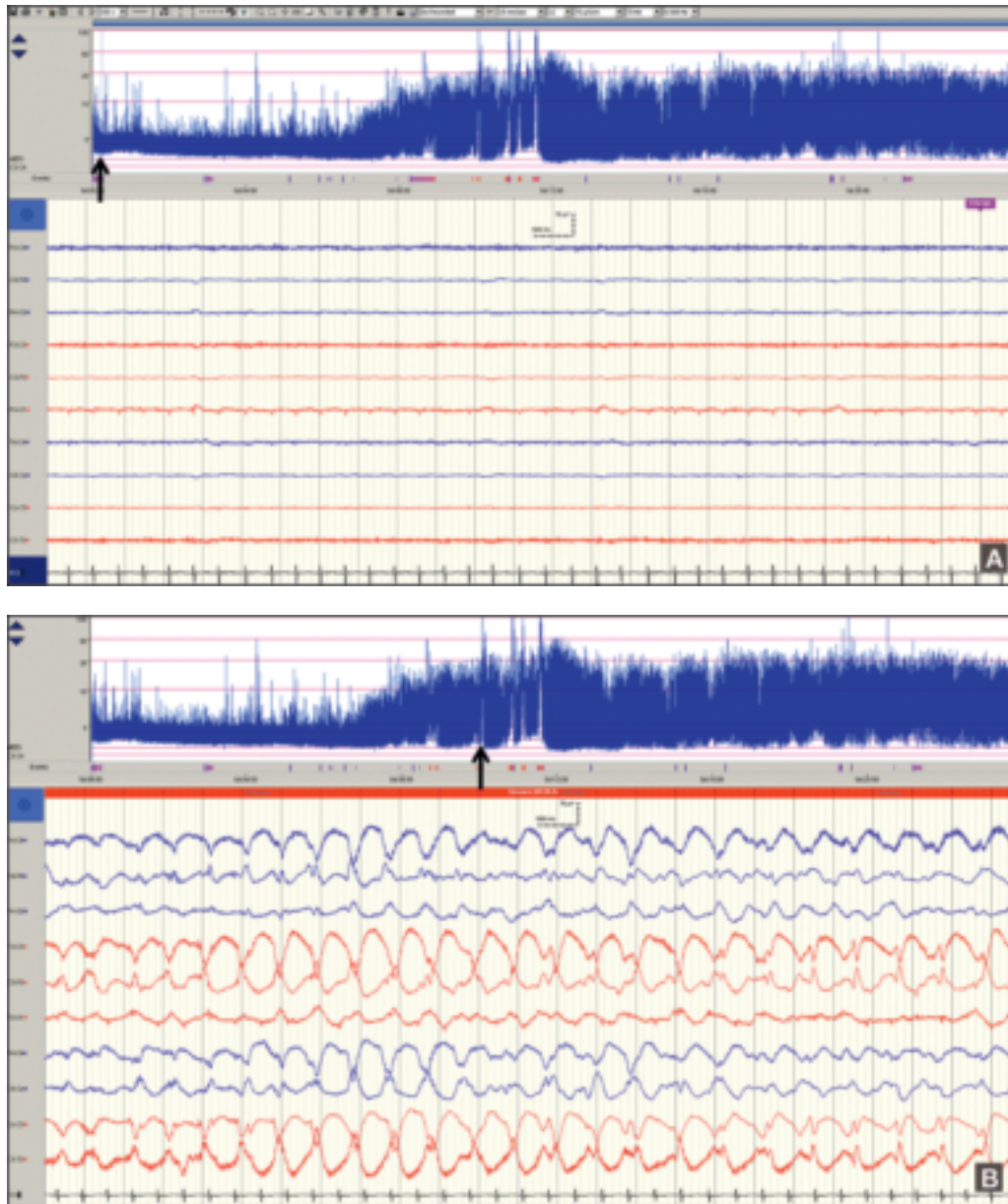


Figure 1. EEG and amplitude-integrated EEG (aEEG) over a 24 hour period from a neonate with poor Apgar scores, cord pH < 7.1 and requiring resuscitation at birth. EEG/aEEG recording commenced when the neonate was three hours old (during the latent phase) while receiving therapeutic hypothermia. Black arrows indicate location of EEG snapshot on aEEG recording.

A: EEG activity was suppressed from the outset and remained suppressed until the neonate was nine hours old.
B: following this period, the EEG began to recover and a burst suppression pattern developed with seizures emerging at 12 hours (probable secondary injury phase) after birth. Seizures responded well to phenobarbitone but a burst suppression background pattern continued.

Seizures occur in moderate and severe HIE only (Sarnat and Sarnat 1976; Levene *et al.*, 1985) and are difficult to control. Studies completed before the widespread use of therapeutic hypothermia show that traditional first and second-line AEDs are often ineffective (Painter *et al.*, 1999; Boylan *et al.*, 2004). There are only a few studies that detail the evolution of electrographic seizure burden in neonates with HIE in the pre-therapeutic hypothermia era (Low *et al.*, 2012; Lynch *et al.*, 2012). Low *et al.* detailed the extensive electrographic seizure burden of neonates with HIE using continuous video-EEG monitoring; the seizure burden in non-cooled neonates was high and status epilepticus common (Figure 2a). Lynch *et al.* (2012) examined the temporal distribution of seizures in neonates with HIE and found that seizures generally have a short period of high electrographic seizure burden followed by a longer period of low seizure burden, resulting in an accumulation of seizures near the time of seizure onset (a positive skew) (Figure 2a and b). Prolonged seizures have been shown to exacerbate pre-existing cerebral damage due to perinatal hypoxic-ischaemia (Yager *et al.*, 2002). Seizures in human neonates with HIE may exacerbate the initial hypoxic-ischaemic injury and require treatment (Miller *et al.*, 2002; Glass *et al.*, 2009; Ancora *et al.*, 2010, Shah *et al.*, 2014). However, this treatment is very difficult to optimize without continuous EEG monitoring.

■ Hypothermia and HIE

The use of cold as a therapeutic agent has had a long and interesting history in both medicine and surgery (Wang *et al.*, 2006; also refer to the chapter by Ikonomidou in this volume). The concept of hypothermia as a treatment for brain injury is not new; its use as a treatment for perinatal asphyxia was suggested over 65 years ago (Miller *et al.*, 1964). In the 1960s, Miller and Westin studied the physiologic basis for the neuroprotective role of hypothermia as a form of treatment for “asphyxia neonatorum”, firstly in newborn animals and then in human newborns (Miller *et al.*, 1964). They demonstrated improved survival without cerebral palsy or mental disability when apnoeic neonates were cooled rapidly after delivery when conventional resuscitation techniques failed. Preliminary studies in adults with coma after resuscitation from out-of-hospital cardiac arrest provided evidence that moderate hypothermia could improve outcomes (Bernard *et al.*, 2002).

Hypothermia delays neuronal depolarisation, decreases the energy requirement for intrinsic cellular support and membrane homeostasis (Nakashima and Todd, 1996; Tooley *et al.*, 2003; Bennet *et al.*, 2007a), reduces cerebral energy metabolism during the primary injury phase, leading to a delay in the progression of primary damage and alleviates post-reperfusion injury. Some studies have shown that cooling markedly delays apoptosis even when it did not completely suppress it (Gunn *et al.*, 2005; Azzopardi *et al.*, 2009a).

■ Therapeutic hypothermia and neonatal seizures

Several experimental animal studies have demonstrated the effects of hypothermia on seizures (Busto *et al.*, 1989; Globus *et al.*, 1995; Nakashima and Todd, 1996; Tooley *et al.*, 2003; Bennet *et al.*, 2007b). In vitro studies have shown that rapidly cooling the cortex to between 20 and 25° C as quickly as possible after seizure onset resulted in a 90% reduction in seizure burden (Hill *et al.*, 2000). In fetal sheep, hypothermia was associated with a marked reduction in the amplitude of seizures in the first 6 hours after a complete umbilical cord occlusion (Bennet *et al.*, 2007b). In a piglet model of asphyxia, the duration of individual electrographic seizures were reduced in a cooled group when compared to a non-cooled group (Tooley *et al.*, 2003). Hypothermia to 30 or 33° C has been shown to

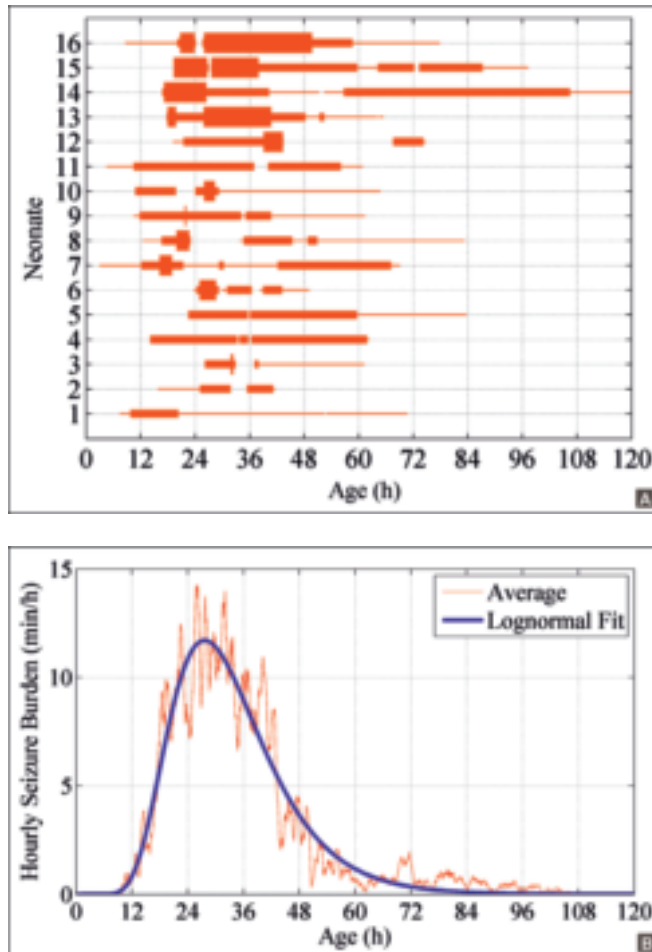


Figure 2. Comparison of the seizure burden between the normothermic and hypothermic neonates with hypoxic-ischaemic encephalopathy (adapted from Low *et al.*, 2012 and Lynch *et al.*, 2012 by Dr Nathan Stevenson).

A and B: at the top (A), a schematic diagram depicting the duration of continuous multichannel EEG monitoring (thin grey horizontal lines) in normothermic neonates with electrographic seizures (thick grey horizontal lines). The temporal distribution of seizures (at the bottom, B) as quantified using a measure of the hourly seizure in typically shows a large positive skew; such that initial seizures generally have a short period of high electrographic seizure burden followed by a period of reducing seizure burden.

inhibit the release of glutamate in a rat model of cerebral ischaemia (Busto *et al.*, 1989). Other effects of hypothermia such as reduced cytotoxic oedema by reducing amino acid release (Nakashima and Todd, 1996) and inhibition of free oxygen radicals (Globus *et al.*, 1995) may reduce seizure burden. Whether the amplitude, morphology and distribution of electrographic seizures in cooled neonates differ to that of non-cooled neonates will require further investigation.

In our recent study of seizures in neonates with hypothermia, we found that seizure burden was reduced in neonates receiving therapeutic hypothermia compared to a normothermic group [60 vs 203 minutes]; and that this was significant in neonates with moderate HIE rather than those with severe HIE (Figure 2c and d) (Low *et al.*, 2012). Our findings were

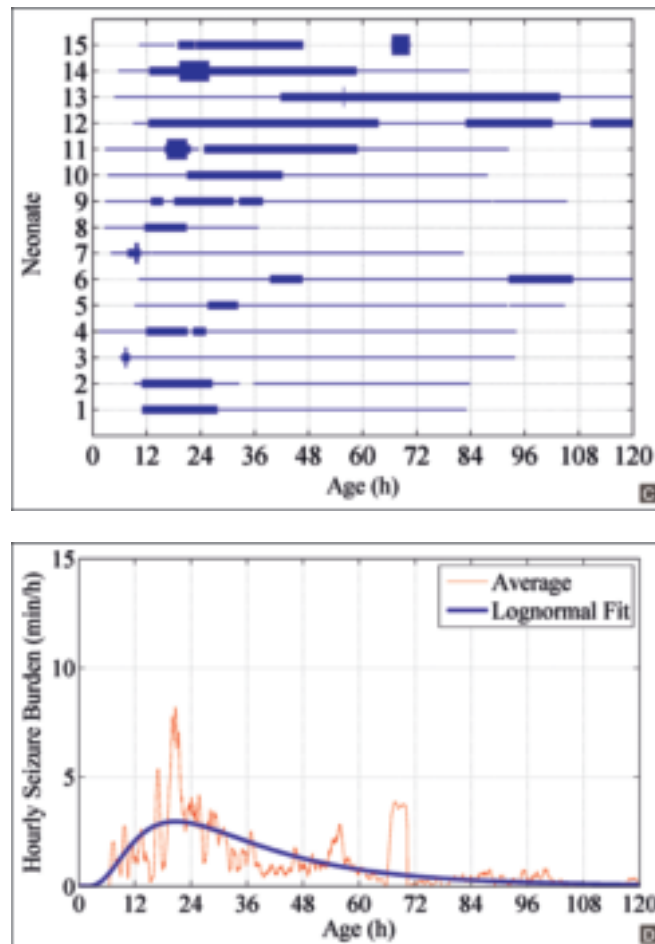


Figure 2 (continued). Comparison of the seizure burden between the normothermic and hypothermic neonates with hypoxic-ischaemic encephalopathy (adapted from Low *et al.*, 2012 and Lynch *et al.*, 2012 by Dr Nathan Stevenson). **C and D:** in the hypothermic group during continuous multichannel EEG monitoring (thin grey horizontal lines), reduced electrographic seizure burden (thick grey horizontal lines) were noted. An altered evolutionary profile, particularly in neonates with moderate rather than in severe hypoxic-ischaemic encephalopathy contributed to this significant reduction in seizure burden in hypothermic neonates. Correspondingly, the hourly seizure burden in this group was also significantly reduced as depicted on the adjacent logarithmic graph.

further confirmed by Srinivasakumar *et al.* who added MRI findings to their study (Srinivasakumar *et al.*, 2013). In neonates with moderate or severe HIE, we found that electrographic seizure rates were almost identical in non-cooled and cooled cohorts (52% and 48% respectively) (Low *et al.*, 2012). These values are consistent with other studies using multichannel EEG (Rafay *et al.*, 2009; Wusthoff *et al.*, 2011; Nash *et al.*, 2011). However, in 2 of the more recent studies (Wusthoff *et al.*, 2011; Nash *et al.*, 2011), the recorded seizure burden was not quantified and a control cohort (non-cooled) was not available for comparison. In another study, even though seizures were less frequent in a cooled group, this was not significantly different when compared to a non-cooled group (Hamelin *et al.*, 2011).

■ Status epilepticus

Few studies have reported the occurrence of status epilepticus during cooling. Status epilepticus occurs in neonates with both moderate and severe HIE (Low *et al.*, 2012). In a cooled cohort studied by Srinivasakumar *et al.*, 5 of 19 neonates with status epilepticus were noted to have severe brain injury on MRI (Srinivasakumar *et al.*, 2013). In another cohort of 56 neonates who were cooled, moderate to severe brain injury (detected by MRI at age of 5 days) was more common in neonates with status epilepticus (Glass *et al.*, 2011). A study by Nash *et al.* also confirmed this finding in 4 of 15 cooled neonates who had status epilepticus and had moderate to severe brain injury (Nash *et al.*, 2011). They concluded that during therapeutic hypothermia, seizures are a risk factor for brain injury, particularly in neonates with status epilepticus. In a study by Wushtoff *et al.*, 23% of neonates undergoing therapeutic hypothermia continued to have status epilepticus (Wushtoff *et al.*, 2011).

Animal studies have advocated the use of therapeutic hypothermia as an adjunct to conventional AEDs to treat status epilepticus (Schmitt *et al.*, 2006). Alternatively, a more effective AED acting as an adjunct to therapeutic hypothermia is much needed to control status epilepticus. Clearly this is an important area for future research, as evidence from small cohort studies shows that neonates undergoing therapeutic hypothermia continue to have periods of status epilepticus which may add further to existing brain injury.

■ Electroclinical dissociation of seizures

Electroclinical dissociation or electroclinical uncoupling is often described in neonatal seizure studies (Boylan *et al.*, 1999; Zangaladze *et al.*, 2008). When this occurs, the clinical signature accompanying the seizure is abolished and seizures are electrographic only. Many neonates exhibit this phenomenon, particularly those with HIE and it is exacerbated by AED use (Connell *et al.*, 1989; Bye and Flanagan, 1995; Scher *et al.*, 2003). A more detailed overview of this subject is beyond the scope of this particular review, but is discussed in greater detail by Boylan *et al.* (Boylan *et al.*, 2013).

Electroclinical dissociation is common in neonates treated with therapeutic hypothermia (Yap *et al.*, 2009; Nash *et al.*, 2011; Wusthoff *et al.*, 2011; Glass *et al.*, 2011). Wusthoff *et al.* report electrographic-only seizures in 47% of neonates treated with therapeutic hypothermia, Nash *et al.* report 43% and Glass *et al.* report 57%. Yap *et al.* monitored a cohort of 20 neonates with selective head cooling (Yap *et al.*, 2009) and monitored seizures with aEEG; they found that 90% of neonates had electrographic-only seizures. The occurrence of electrographic-only seizures in this study is higher than most reports and may reflect the use of specific AED protocols in this population.

■ Antiepileptic drugs (AEDs) and therapeutic hypothermia

During both the pre and post-therapeutic hypothermia era, phenobarbitone remains the most commonly used first-line AED in most neonatal units worldwide (Bartha *et al.*, 2007; Vento *et al.*, 2010) and has been shown to be effective in treating approximately 50% of neonatal seizures (Painter *et al.*, 1999; Boylan *et al.*, 2002; Booth and Evans, 2004). The reduced efficacy of this GABA-enhancing AED has been linked to altered neuronal chloride transport in the developing brain (Dzhala *et al.*, 2005).

Based on multicentre studies in the United States (Bartha *et al.*, 2007) and in Europe (Vento *et al.*, 2010), there is still no consensus on a standard protocol for the use of AEDs in neonatal seizures. We have previously shown that there was no significant difference between non-cooled and cooled HIE groups with respect to the number, dose and age in hours when first and second-line AEDs were administered (Low *et al.*, 2012). In a rodent study, phenobarbitone was shown to augment the therapeutic effect of cooling (Barks *et al.*, 2010). As an AED, phenobarbitone has the potential to reduce endogenous heat production and thus exaggerate the fall in temperature during active cooling.

It is known that the half-life of phenobarbitone is significantly increased when neonates are treated with hypothermia (Filippi *et al.*, 2011) and with reduced hepatic metabolism during hypothermia, plasma drug levels will accumulate (Roka *et al.*, 2008). The bioavailability of phenobarbitone in neonates can range from 45 to 500 hours (Takemoto, 2012); it can be variable depending on circumstances (Filippi *et al.*, 2011; van den Broek *et al.*, 2012; Shellhaas *et al.*, 2013) and is different from adults (Marsot *et al.*, 2013). Van den Broek *et al.* assessed the pharmacokinetics of phenobarbitone in a cohort of 31 neonates (≥ 36 weeks gestation) with HIE who were cooled (van den Broek *et al.*, 2012). The authors advocate the use of up to 40 mg/kg of phenobarbitone in total before proceeding to a second-line AED as plasma levels of phenobarbitone remained below therapeutic range during therapeutic hypothermia. Based on a study undertaken before the era of therapeutic hypothermia, phenobarbitone doses higher than 40 mg/kg have been shown to increase neuronal apoptosis (Gilman *et al.*, 1989).

■ Seizures during rewarming following therapeutic hypothermia

Seizures have been reported in the rewarming period following therapeutic hypothermia (Battin *et al.*, 2004; Kendall *et al.*, 2012; Shah *et al.*, 2014). In a rabbit model cooled to a core temperature of 33° C, a decrease in nitric oxide production and hippocampal cell loss were noted during kainate-induced seizures (Takei *et al.*, 2005). During rewarming, there was an increase in nitric oxide production in the hippocampus during seizures. Transient rebound epileptiform activity has previously been observed when hypothermia was discontinued after 72 hours (Gunn *et al.*, 2005). Although rewarming seizures have been anecdotally reported (Battin *et al.*, 2004; Gerrits *et al.*, 2005; Shah *et al.*, 2014), they can continue unabated even after the rewarming period has completed (Kendall *et al.*, 2012). Shah *et al.* recently showed that in human term neonates, seizures are commonly seen during cooling and a significant second peak of seizures during the rewarming period is not uncommon (Shah *et al.*, 2014). On recommencing therapeutic hypothermia immediately after a period of rewarming, seizures that re-emerge during rewarming can abate without the use of any AED (Kendall *et al.*, 2012).

In our study, seizures were seen in four of 15 cooled neonates when therapeutic hypothermia was discontinued (Low *et al.*, 2012). Two of the 4 cases had a shorter duration of therapeutic hypothermia as a decision was made to redirect in neonatal intensive care. In the remaining 2 neonates, electrographic seizures were observed following discontinuation of therapeutic hypothermia despite the fact that therapeutic hypothermia started at 6 and 9 hours respectively, after birth and continued for 72 hours. The incidence of rewarming seizures remains speculative. Now that EEG monitoring is continuing during the rewarming period, more studies describing the re-emergence of seizures during rewarming may be reported. Although some studies have speculated that rewarming seizures are benign (Battin *et al.*, 2004; Gerrits *et al.*, 2005; Shah *et al.*, 2014), further studies are required to establish their significance.

■ Therapeutic hypothermia trials and seizures

Neonatal outcome studies have shown that seizures are powerful predictors of death or permanent neurodisability (Pisani *et al.*, 2008; Glass *et al.*, 2009). Previously published neonatal hypothermia trials could not accurately measure seizure burden as their protocols did not include prolonged continuous multichannel EEG monitoring. These studies used clinical (Kwon *et al.*, 2011) and/or aEEG monitoring (Simbruner *et al.*, 2010; Edwards *et al.*, 2010) for seizure recognition. The recently published Neonatal Research Network Whole Body Hypothermia Trial relied on clinical recognition of seizures only (Kwon *et al.*, 2011) and when the authors adjusted for hypothermia and severity of encephalopathy, hypothermia did not appear to have any impact on the frequency of clinical seizures and outcome. However, clinical estimation of seizure burden is notoriously unreliable with the majority of neonatal seizures being subclinical or electrographic only (Murray *et al.*, 2008; Malone *et al.*, 2009).

When available in some participating neonatal institutions in the TOBY trial, the aEEG was used for recruitment and as a monitoring tool during therapeutic hypothermia (Azzopardi *et al.*, 2009b). At recruitment, clinical seizures and seizures detected by aEEG were present in 67% (74/110) and 29% (33/115) of neonates respectively. The trial considered seizures as a complication during therapeutic hypothermia, with a decreasing incidence from day one to four (90% to 23%). Both clinical recognition of seizures and the aEEG are known to both over, and under estimate the true seizure burden (Murray *et al.*, 2008). In addition, the aEEG cannot detect short seizures, seizures that do not generalize and low voltage seizures.

At present, the therapeutic hypothermia registry lead by Azzopardi *et al.* has not made brain monitoring a prerequisite for cooling (Azzopardi *et al.*, 2007). It was recommended that if possible, some form of cerebral function monitoring should be performed on neonates receiving therapeutic hypothermia either before the induction of cooling or as soon as possible during cooling. We strongly support the view that EEG monitoring is crucial during therapeutic hypothermia and also believe that monitoring should be extended after cooling has been discontinued as seizures may emerge during rewarming.

Unfortunately continuous EEG monitoring is hard to maintain in the neonatal intensive care unit and a specialized team is required for interpretation, which is rarely available. Many centres have now implemented remote monitoring of the EEG by specialized teams but this is expensive and time consuming. A more promising option is in the form of automated seizure detection using specially trained and validated algorithms. Research is ongoing and a number of excellent algorithms have been described for neonates using off-line data analysis. Few to date have been implemented routinely in the neonatal intensive care unit with the notable exception of the BrainZ aEEG monitoring system. One clinical validation trial is currently underway in Europe (ANSeR– Algorithm for Neonatal Seizure Recognition <<http://clinicaltrials.gov/show/NCT02160171>>) which may provide useful information on the utility of automated seizure detection for term neonates with HIE.

■ Conclusion

Seizures are common in neonates with HIE who are treated with therapeutic hypothermia. While the number of neonates with seizures is similar in both normothermic and hypothermic groups, the overall seizure burden has reduced during therapeutic hypothermia.

This is particularly evident in neonates with moderate encephalopathy where current therapeutic hypothermia strategies seem to have the greatest benefit. It is not known if this reduced seizure burden contributes to the increased benefit seen following therapeutic hypothermia in moderate encephalopathy, or if it is simply a reflection of reduced neuronal damage during therapeutic hypothermia. Only large multicentre studies using continuous multichannel EEG monitoring in neonates with HIE undergoing therapeutic hypothermia will be able to answer this important question.

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References

- Aly H, Mohsen L, Badrawi N, Gabr H, Ali Z, Akmal D. Viability and neural differentiation of mesenchymal stem cells derived from the umbilical cord following perinatal asphyxia. *J Perinatol* 2012; 32: 671-76.
- Ancora G, Soffritti S, Lodi R, *et al.* A combined a-EEG and MR spectroscopy study in term newborns with hypoxic-ischemic encephalopathy. *Brain Dev* 2010; 32: 835-42.
- Azzopardi D, Brocklehurst P, Currie A, Edwards D. UK TOBY Cooling Register Protocol Version 3 [online]. <https://www.npeu.ox.ac.uk/tobyregister>. 2007.
- Azzopardi DV, Strohm B, Edwards AD, *et al.* Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N Engl J Med* 2009; 361: 1349-58.
- Azzopardi D, Strohm B, Edwards AD, *et al.* Treatment of asphyxiated newborns with moderate hypothermia in routine clinical practice: how cooling is managed in the UK outside a clinical trial. *Arch Dis Child Fetal Neonatal Ed* 2009; 94: F260-64.
- Azzopardi D, Strohm B, Marlow N, *et al.* Effects of hypothermia for perinatal asphyxia on childhood outcomes. *N Engl J Med* 2014; 371: 140-49.
- Barks JD, Liu YQ, Shangguan Y, Silverstein FS. Phenobarbital augments hypothermic neuroprotection. *Pediatr Res* 2010; 67: 532-37.
- Barthä AI, Shen J, Katz KH, *et al.* Neonatal seizures: multicenter variability in current treatment practices. *Pediatr Neurol* 2007; 37: 85-90.
- Battin M, Bennet L, Gunn AJ. Rebound seizures during rewarming. *Pediatrics* 2004; 114: 1369.
- Bennet L, Roelfsema V, Pathipati P, Quaedackers JS, Gunn AJ. Relationship between evolving epileptiform activity and delayed loss of mitochondrial activity after asphyxia measured by near-infrared spectroscopy in preterm fetal sheep. *J Physiol* 2006; 572 (Pt 1): 141-54.
- Bennet L, Roelfsema V, George S, Dean JM, Emerald BS, Gunn AJ. The effect of cerebral hypothermia on white and grey matter injury induced by severe hypoxia in preterm fetal sheep. *J Physiol* 2007; 578 (Pt 2): 491-506.
- Bennet L, Dean JM, Wassink G, Gunn AJ. Differential effects of hypothermia on early and late epileptiform events after severe hypoxia in preterm fetal sheep. *J Neurophysiol* 2007; 97(1): 572-78.
- Bernard SA, Gray TW, Buist MD, *et al.* Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *N Engl J Med* 2002; 346: 557-63.
- Bjorkman ST, Miller SM, Rose SE, Burke C, Colditz PB. Seizures are associated with brain injury severity in a neonatal model of hypoxia-ischemia. *Neuroscience* 2010; 166: 157-67.
- Booth D, Evans DJ. Anticonvulsants for neonates with seizures. *Cochrane Database Syst Rev* 2004: CD004218.

- Boylan GB, Pressler RM, Rennie JM, *et al.* Outcome of electroclinical, electrographic, and clinical seizures in the newborn infant. *Dev Med Child Neurol* 1999; 41: 819-25.
- Boylan GB, Rennie JM, Pressler RM, Wilson G, Morton M, Binnie CD. Phenobarbitone, neonatal seizures, and video-EEG. *Arch Dis Child Fetal Neonatal Ed* 2002; 86: F165-70.
- Boylan GB, Rennie JM, Chorley G, *et al.* Second-line anticonvulsant treatment of neonatal seizures: a video-EEG monitoring study. *Neurology* 2004; 62(3): 486-88.
- Boylan GB, Stevenson NJ, Vanhatalo S. Monitoring neonatal seizures. *Semin Fetal Neonatal Med* 2013; 18: 202-8.
- Busto R, Globus MY, Dietrich WD, Martinez E, Valdes I, Ginsberg MD. Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain. *Stroke* 1989; 20: 904-10.
- Bye A, Flanagan D. Electroencephalograms, clinical observations and the monitoring of neonatal seizures. *J Paediatr Child Health* 1995; 31: 503-7.
- Charriaut-Marlangue C, Nguyen T, Bonnin P, *et al.* Sildenafil mediates blood-flow redistribution and neuroprotection after neonatal hypoxia-ischemia. *Stroke* 2014; 45: 850-6.
- Connell J, Oozeer R, de VL, Dubowitz LM, Dubowitz V. Clinical and EEG response to anticonvulsants in neonatal seizures. *Arch Dis Child* 1989; 64 (4 Spec No): 459-64.
- Dzhala VI, Talos DM, Sdrulla DA *et al.* NKCC1 transporter facilitates seizures in the developing brain. *Nat Med* 2005; 11(11): 1205-13.
- Edwards AD, Brocklehurst P, Gunn AJ, *et al.* Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data. *BMJ* 2010; 340: c363.
- Fan X, van Bel F. Pharmacological neuroprotection after perinatal asphyxia. *J Matern Fetal Neonatal Med* 2010; 23 (suppl 3): 17-19.
- Faulkner S, Bainbridge A, Kato T, *et al.* Xenon augmented hypothermia reduces early lactate/N-acetylaspartate and cell death in perinatal asphyxia. *Ann Neurol* 2011; 70: 133-50.
- Filippi L, la MG, Cavallaro G, *et al.* Phenobarbital for neonatal seizures in hypoxic ischemic encephalopathy: a pharmacokinetic study during whole body hypothermia. *Epilepsia* 2011; 52: 794-801.
- Gerrits LC, Battin MR, Bennet L, Gonzalez H, Gunn AJ. Epileptiform activity during rewarming from moderate cerebral hypothermia in the near-term fetal sheep. *Pediatr Res* 2005; 57: 342-46.
- Gilman JT, Gal P, Duchowny MS, Weaver RL, Ransom JL. Rapid sequential phenobarbital treatment of neonatal seizures. *Pediatrics* 1989; 83: 674-78.
- Glass HC, Glidden D, Jeremy RJ, Barkovich AJ, Ferriero DM, Miller SP. Clinical neonatal seizures are independently associated with outcome in infants at risk for hypoxic-ischemic brain injury. *J Pediatr* 2009; 155: 318-23.
- Glass HC, Nash KB, Bonifacio SL, *et al.* Seizures and magnetic resonance imaging-detected brain injury in newborns cooled for hypoxic-ischemic encephalopathy. *J Pediatr* 2011; 159: 731-35.
- Glass HC, Wusthoff CJ, Shellhaas RA. Amplitude-integrated electro-encephalography: the child neurologist's perspective. *J Child Neurol* 2013; 28: 1342-50.
- Globus MY, Alonso O, Dietrich WD, Busto R, Ginsberg MD. Glutamate release and free radical production following brain injury: effects of posttraumatic hypothermia. *J Neurochem* 1995; 65: 1704-11.
- Gunn AJ, Parer JT, Mallard EC, Williams CE, Gluckman PD. Cerebral histologic and electrocorticographic changes after asphyxia in fetal sheep. *Pediatr Res* 1992; 31: 486-91.
- Gunn AJ, Battin M, Gluckman PD, Gunn TR, Bennet L. Therapeutic hypothermia: from lab to NICU. *J Perinat Med* 2005; 33: 340-46.
- Gunn AJ, Thoresen M. Hypothermic neuroprotection. *NeuroRx* 2006; 3: 154-69.

- Hamelin S, Delnard N, Cneude F, Debillon T, Vercueil L. Influence of hypothermia on the prognostic value of early EEG in full-term neonates with hypoxic ischemic encephalopathy. *Neurophysiol Clin* 2011; 41: 19-27.
- Harris MN, Carey WA, Ellsworth MA, *et al.* Perceptions and practices of therapeutic hypothermia in American neonatal intensive care units. *Am J Perinatol* 2014; 31: 15-20.
- Herrera EA, Macchiavello R, Montt C, *et al.* Melatonin improves cerebrovascular function and decreases oxidative stress in chronically hypoxic lambs. *J Pineal Res* 2014 May 8. [Epub ahead of print].
- Hill MW, Wong M, Amarakone A, Rothman SM. Rapid cooling aborts seizure-like activity in rodent hippocampal-entorhinal slices. *Epilepsia* 2000; 41: 1241-48.
- Jacobs SE, Berg M, Hunt R, Tarnow-Mordi WO, Inder TE, Davis PG. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev* 2013; 1: CD003311.
- Kendall GS, Mathieson S, Meek J, Rennie JM. Recooling for rebound seizures after rewarming in neonatal encephalopathy. *Pediatrics* 2012; 130: e451-55.
- Kwon JM, Guillet R, Shankaran, *et al.* Clinical seizures in neonatal hypoxic-ischemic encephalopathy have no independent impact on neurodevelopmental outcome: secondary analyses of data from the neonatal research network hypothermia trial. *J Child Neurol* 2011; 26: 322-28.
- Lawn JE, Kerber K, Enweronu-Laryea C, Cousens S. 3.6 million neonatal deaths – what is progressing and what is not? *Semin Perinatol* 2010; 34: 371-86.
- Levene ML, Kornberg J, Williams TH. The incidence and severity of post-asphyxial encephalopathy in full-term infants. *Early Hum Dev* 1985; 11: 21-26.
- Lorek A, Takei Y, Cady EB, *et al.* Delayed (“secondary”) cerebral energy failure after acute hypoxia-ischemia in the newborn piglet: continuous 48-hour studies by phosphorus magnetic resonance spectroscopy. *Pediatr Res* 1994; 36: 699-706.
- Low E, Boylan GB, Mathieson SR, *et al.* Cooling and seizure burden in term neonates: an observational study. *Arch Dis Child Fetal Neonatal Ed* 2012; 97: F267-72.
- Lynch NE, Stevenson NJ, Livingstone V, Murphy BP, Rennie JM, Boylan GB. The temporal evolution of electrographic seizure burden in neonatal hypoxic ischemic encephalopathy. *Epilepsia* 2012; 53: 549-57.
- Malone A, Ryan CA, Fitzgerald A, Burgoyne L, Connolly S, Boylan GB. Interobserver agreement in neonatal seizure identification. *Epilepsia* 2009; 50: 2097-101.
- Marlow N, Budge H. Prevalence, causes, and outcome at 2 years of age of newborn encephalopathy. *Arch Dis Child Fetal Neonatal Ed* 2005; 90: F193-94.
- Marsot A, Brevaut-Malaty V, Vialet R, Boulamery A, Bruguerolle B, Simon N. Pharmacokinetics and absolute bioavailability of phenobarbital in neonates and young infants, a population pharmacokinetic modelling approach. *Fundam Clin Pharmacol* 2014; 28: 465-71.
- Miller JA Jr, Miller FS, Westin B. Hypothermia in the treatment of asphyxia neonatorum. *Biol Neonat* 1964; 6: 148-63.
- Miller SP, Weiss J, Barnwell A, *et al.* Seizure-associated brain injury in term newborns with perinatal asphyxia. *Neurology* 2002; 58: 542-8.
- Murray DM, Boylan GB, Ali I, Ryan CA, Murphy BP, Connolly S. Defining the gap between electrographic seizure burden, clinical expression and staff recognition of neonatal seizures. *Arch Dis Child Fetal Neonatal Ed* 2008; 93: F187-91.
- Nakashima K, Todd MM. Effects of hypothermia on the rate of excitatory amino acid release after ischemic depolarization. *Stroke* 1996; 27(5): 913-18.
- Nash KB, Bonifacio SL, Glass HC, *et al.* Video-EEG monitoring in newborns with hypoxic-ischemic encephalopathy treated with hypothermia. *Neurology* 2011; 76: 556-62.
- National Institute for Health and Clinical Excellence (NICE). Controlled cooling to treat newborn babies with brain injury caused by oxygen shortage during birth. Information about NICE procedure guidance. 347; Reference No N2184 [online]. www.nice.org.uk. 2010.

- Painter MJ, Scher MS, Stein AD, *et al.* Phenobarbital compared with phenytoin for the treatment of neonatal seizures. *N Engl J Med* 1999; 341: 485-89.
- Pisani F, Copioli C, Di GC, Turco E, Sisti L. Neonatal seizures: relation of ictal video-electroencephalography (EEG) findings with neurodevelopmental outcome. *J Child Neurol* 2008; 23: 394-98.
- Rafay MF, Cortez MA, de Veber GA, *et al.* Predictive value of clinical and EEG features in the diagnosis of stroke and hypoxic ischemic encephalopathy in neonates with seizures. *Stroke* 2009; 40: 2402-07.
- Robertson NJ, Faulkner S, Fleiss B, *et al.* Melatonin augments hypothermic neuroprotection in a perinatal asphyxia model. *Brain* 2013; 136: 90-105.
- Roka A, Melinda KT, Vasarhelyi B, Machay T, Azzopardi D, Szabo M. Elevated morphine concentrations in neonates treated with morphine and prolonged hypothermia for hypoxic ischemic encephalopathy. *Pediatrics* 2008; 121: e844-49.
- Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol* 1976; 33: 696-705.
- Schmitt FC, Buchheim K, Meierkord H, Holtkamp M. Anticonvulsant properties of hypothermia in experimental status epilepticus. *Neurobiol Dis* 2006; 23: 689-96.
- Scher MS, Alvin J, Gaus L, Minnigh B, Painter MJ. Uncoupling of EEG-clinical neonatal seizures after antiepileptic drug use. *Pediatr Neurol* 2003; 28: 277-80.
- Scher MS, Steppe DA, Beggarly M. Timing of neonatal seizures and intrapartum obstetrical factors. *J Child Neurol* 2008; 23: 640-43.
- Schiering IA, de Haan TR, Niermeijer JM, *et al.* Correlation between clinical and histologic findings in the human neonatal hippocampus after perinatal asphyxia. *J Neuropathol Exp Neurol* 2014; 73: 324-34.
- Shah DK, Wusthoff CJ, Clarke P, *et al.* Electrographic seizures are associated with brain injury in newborns undergoing therapeutic hypothermia. *Arch Dis Child Fetal Neonatal Ed* 2014; 99: F219-24.
- Shellhaas RA, Soaita AI, Clancy RR. Sensitivity of amplitude-integrated electroencephalography for neonatal seizure detection. *Pediatrics* 2007; 120: 770-7.
- Shellhaas RA, Ng CM, Dillon CH, Barks JD, Bhatt-Mehta V. Population pharmacokinetics of phenobarbital in infants with neonatal encephalopathy treated with therapeutic hypothermia. *Pediatr Crit Care Med* 2013; 14: 194-202.
- Simbruner G, Mittal RA, Rohlmann F, Muche R. Systemic hypothermia after neonatal encephalopathy: outcomes of neoNEURO.network RCT. *Pediatrics* 2010; 126: e771-78.
- Srinivasakumar P, Zempel J, Wallendorf M, Lawrence R, Inder T, Mathur A. Therapeutic hypothermia in neonatal hypoxic ischemic encephalopathy: electrographic seizures and magnetic resonance imaging evidence of injury. *J Pediatr* 2013; 163: 465-70.
- Takei Y, Sunohara D, Nishikawa Y, *et al.* Effects of rapid rewarming on cerebral nitric oxide production and cerebral hemodynamics after hypothermia therapy for kainic acid-induced seizures in immature rabbits. *Pediatr Int* 2005; 47: 53-9.
- Takemoto CK, Hodding JH, Kraus DM. *The Pediatric Dosage Handbook*, 19th ed. Hudson, Ohio: Lexi-Comp, Inc, 2012.
- Tan WK, Williams CE, During MJ, *et al.* Accumulation of cytotoxins during the development of seizures and edema after hypoxic-ischemic injury in late gestation fetal sheep. *Pediatr Res* 1996; 39: 791-7.
- Tooley JR, Satas S, Porter H, Silver IA, Thoresen M. Head cooling with mild systemic hypothermia in anesthetized piglets is neuroprotective. *Ann Neurol* 2003; 53: 65-72.
- van den Broek MP, Groenendaal F, Toet MC, *et al.* Pharmacokinetics and clinical efficacy of phenobarbital in asphyxiated newborns treated with hypothermia: a thermopharmacological approach. *Clin Pharmacokinet* 2012; 51: 671-9.

- Vento M, de Vries LS, Alberola A *et al.* Approach to seizures in the neonatal period: a European perspective. *Acta Paediatr* 2010; 99(4): 497-501.
- Volpe JJ. *Neurology of the Newborn*. Philadelphia, PA: WB Saunders Company, 2008.
- Wang H, Olivero W, Wang D, Lanzino G. Cold as a therapeutic agent. *Acta Neurochir (Wien)* 2006; 148(5): 565-70.
- Wassink G, Gunn ER, Drury PP, Bennet L, Gunn AJ. The mechanisms and treatment of asphyxial encephalopathy. *Front Neurosci* 2014; 8: 40.
- Wirrell EC, Armstrong EA, Osman LD, Yager JY. Prolonged seizures exacerbate perinatal hypoxic-ischemic brain damage. *Pediatr Res* 2001; 50: 445-54.
- Wusthoff CJ, Dlugos DJ, Gutierrez-Colina A, *et al.* Electrographic seizures during therapeutic hypothermia for neonatal hypoxic-ischemic encephalopathy. *J Child Neurol* 2011; 26: 724-8.
- Yager JY, Armstrong EA, Miyashita H, Wirrell EC. Prolonged neonatal seizures exacerbate hypoxic-ischemic brain damage: correlation with cerebral energy metabolism and excitatory amino acid release. *Dev Neurosci* 2002; 24: 367-81.
- Yap V, Engel M, Takenouchi T, Perlman JM. Seizures are common in term infants undergoing head cooling. *Pediatr Neurol* 2009; 41: 327-31.
- Zangaladze A, Nei M, Liporace JD, Sperling MR. Characteristics and clinical significance of subclinical seizures. *Epilepsia* 2008; 49: 2016-21.

Cooling and seizure burden in term neonates: an observational study

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ABSTRACT

Objective To investigate any possible effect of cooling on seizure burden, the authors quantified the recorded electrographic seizure burden based on multichannel video-EEG recordings in term neonates with hypoxic-ischaemic encephalopathy (HIE) who received cooling and in those who did not.

Study design Retrospective observational study.

Patients Neonates >37 weeks gestation born between 2003 and 2010 in two hospitals.

Methods Off-line analysis of prolonged continuous multichannel video-EEG recordings was performed independently by two experienced encephalographers. Comparison between the recorded electrographic seizure burden in non-cooled and cooled neonates was assessed. Data were treated as non-parametric and expressed as medians with interquartile ranges (IQR).

Results One hundred and seven neonates with HIE underwent prolonged continuous multichannel EEG monitoring. Thirty-seven neonates had electrographic seizures, of whom 31 had EEG recordings that were suitable for the analysis (16 non-cooled and 15 cooled). Compared with non-cooled neonates, multichannel EEG monitoring commenced at an earlier postnatal age in cooled neonates (6 (3–9) vs 15 (5–20) h) and continued for longer (88 (75–101) vs 55 (41–60) h). Despite this increased opportunity to capture seizures in cooled neonates, the recorded electrographic seizure burden in the cooled group was significantly lower than in the non-cooled group (60 (39–224) vs 203 (141–406) min). Further exploratory analysis showed that the recorded electrographic seizure burden was only significantly reduced in cooled neonates with moderate HIE (49 (26–89) vs 162 (97–262) min).

Conclusions A decreased seizure burden was seen in neonates with moderate HIE who received cooling. This finding may explain some of the therapeutic benefits of cooling seen in term neonates with moderate HIE.

INTRODUCTION

Approximately, 50–75% of neonatal seizures at term are attributable to neonatal hypoxic-ischaemic encephalopathy (HIE).¹ Neonatal outcome studies have shown that seizures are powerful predictors of death or permanent neurological disability.^{2–3} However, these studies relied almost entirely on the detection of seizures using clinical criteria² or amplitude-integrated (aEEG).³ It is well known that clinical assessment⁴ and aEEG^{5–7} can miss many seizures and therefore cannot accurately quantify the precise seizure burden in neonates. Accurate identification and quantification of neonatal seizures require continuous multichannel video-EEG monitoring.

What is already known on this topic

- Cooling has been shown to reduce the combined rate of death and disability at 18 months of age in term neonates with hypoxic-ischaemic encephalopathy.
- Early, prolonged and continuous multichannel EEG provides accurate identification and quantification of electrographic seizure burden in term neonates.

What this study adds

- The seizure burden was less in a group of neonates treated with therapeutic hypothermia compared with a similar group who were not cooled.
- This is the first study using prolonged continuous multichannel EEG to quantify the seizure burden in non-cooled and cooled term neonates with hypoxic-ischaemic encephalopathy.

The evidence of benefit is considered sufficient for the National Institute for Health and Clinical Excellence to endorse the use of cooling for hypoxic perinatal brain injury in the UK.⁸ A meta-analysis of three trials which enrolled 767 neonates showed that cooling reduced the combined rate of death or disability at 18 months.⁷ However, the precise mechanism by which cooling achieves neuroprotection in neonates with HIE is unknown. In the biphasic model of neuronal death following hypoxic injury, the cascade of events which occurs in the secondary reperfusion phase may be associated with seizures, an accumulation of cytotoxins and the failure of oxidative cerebral metabolism.^{9–10} Cooling may reduce seizure burden in neonates by affecting some mechanisms during this vital phase of brain injury.

We aimed to determine whether cooling was associated with a reduction in seizure burden in HIE neonates by quantifying seizure burden using continuous multichannel video-EEG monitoring.

PATIENT AND METHODS

Non-cooled neonates were enrolled between June 2003 to September 2006 and January 2009 to March 2010 from Cork University Maternity

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Hospital (CUMH), Ireland. Cooled neonates were enrolled between January 2009 and September 2010 from CUMH and University College London Hospitals (UCLH), UK. Neonates >37 weeks gestation with HIE were enrolled for EEG monitoring if they fulfilled ≥ 2 of the following criteria: Apgar score <6 at 5 min, a continued need for resuscitation after birth, clinical evidence of encephalopathy or seizures within 24 h of birth. At both hospitals, every neonate was assigned a clinical grade of encephalopathy using the modified Sarnat score at 24 h of age.¹¹ This study was conducted with the approval from the Clinical Research Ethics Committees of the Cork Teaching hospitals, Ireland, and the National Health Service in the UK, via the Integrated Research Application Service. Written, informed consent was obtained from at least one parent of each neonate who participated in this study.

Neonates were cooled according to the entry criteria and guidelines set by the UK Total Body Hypothermia for Neonatal Encephalopathy (TOBY) cooling registry (from the *UK TOBY Cooling Register Clinician's Handbook*, section 2.1, <http://www.npeu.ox.ac.uk/toby>). Either the Tecotherm TS med 200

(Tec-Com, Halle, Germany) or the CritiCool MTRE machine (Charter Kontron, Milton Keynes, UK) was used. Neonates were cooled to a rectal temperature of 33–34°C for 72 h (unless contraindicated) and were slowly rewarmed. Within both hospitals, treatment was based on clinical observation and EEG findings. Both groups had continuous EEG monitoring but the neonatologists did not interpret the EEG recordings. On our monitoring system, the aEEG and the multichannel EEG were simultaneously recorded and many of our neonatologists would have used the aEEG as an aid to clinical decision-making. All clinical seizures were treated. The aEEG was used to confirm clinically suspected seizures. If they were concerned about any abnormal clinical behaviours or aEEG patterns, the encephalographers would be asked to interpret the multichannel EEG at a later stage. Immediate reporting of the multichannel EEG was not available, so that aEEG and clinical suspicion were the mainstays of seizure diagnosis. Phenobarbitone was the first-line anticonvulsant administered to a maximum dose of 40 mg/kg intravenously. Second-line anticonvulsants were administered if clinical and/or electrographic seizures recurred following

Table 1 Clinical characteristics of the neonates included in the study

| | Non-cooled (n=16) | Cooled (n=15) | p Value |
|---|---------------------|---------------------|---------|
| Gestational age (weeks) | 41 (40–41) | 40 (40–41) | 0.300 |
| Birth weight (g) | 3488 (3163–3733) | 3275 (3000–4130) | 0.707 |
| Gender (male:female) | 10:6 | 9:6 | 0.886* |
| Clinical Sarnat score | | | |
| Moderate | 6 | 8 | 0.376* |
| Severe | 10 | 7 | |
| 5-min Apgar score | 6 (2–8) | 4 (2–4) | 0.050 |
| First pH | 7.134 (7.032–7.217) | 6.930 (6.800–7.100) | 0.009 |
| Number of anticonvulsants | 2 (1–3) | 1 (1–2) | 0.274 |
| First-line anticonvulsant (age in h) | 12 (9–19) | 14 (10–24) | 0.504 |
| Total dose of first-line anticonvulsant (mg/kg) | 30 (20–40) | 20 (20) | 0.203 |
| Second-line anticonvulsant (age in h) | 28 (24–31) | 26 (19–38) | 0.556 |
| Number of neonates on morphine | 8 | 15 | 0.002* |

Data are median (IQR) or n.

* χ^2 test for the proportion of gender and clinical Sarnat score for neonatal hypoxia-ischaemic encephalopathy in non-cooled and cooled groups.

Table 2 Individual characteristics of non-cooled neonates with hypoxia-ischaemic encephalopathy

| Case | Clinical Sarnat | Recorded seizure burden (min) | Seizure number (n) | Mean seizure duration (s) | Age at first EEG seizure | Age at first-line anticonvulsant | Time from EEG seizure onset to treatment | Second-line anticonvulsant | | Other drugs |
|---------------------|-----------------|-------------------------------|--------------------|---------------------------|--------------------------|----------------------------------|--|----------------------------|---------------|-------------|
| | | | | | | | | Age | Total dose | |
| C1 ^S | 2 | 38 | 4 | 574 | 10 h 15 m | N | N | | | M |
| C2 | 3 | 106 | 43 | 147 | 18 h | | | 23 h 14 m | Pt=10 mg/kg | M |
| C3 ^S | 2 | 116 | 21 | 331 | 26 h 11 m | 8 h 5 m | B | 25 h 5 m | Pt=20 mg/kg | |
| C4 ^S | 3 | 137 | 84 | 98 | 14 h 12 m | N | N | | | M, Tr |
| C5 ^S | 2 | 152 | 99 | 92 | 22 h 30 m | 22 h 34 m | 4 m | 32 h 44 m | Pt=20 mg/kg | |
| C6 ^{E, S} | 2 | 172 | 21 | 493 | 25 h 30 m | 19 h 13 m | B | 28 h 28 m | Pt=20 mg/kg | |
| C7 ^E | 3 | 183 | 121 | 91 | 12 h 40 m | 12 h 20 m | B | 35 h 35 m | Mz=100 mcg/kg | M |
| C8 ^E | 3 | 199 | 41 | 291 | 17 h 7 m | 10 h 50 m | B | | | M |
| C9 | 3 | 206 | 60 | 206 | 12 h 20 m | 24 h 24 m | 12 h 4 m | | | M |
| C10 ^{E, S} | 2 | 212 | 66 | 193 | 10 h 54 m | 10 h 10 m | B | 28 h 40 m | Pt=20 mg/kg | |
| C11 | 3 | 239 | 150 | 96 | 10 h 56 m | 19 h 35 m | 8 h 39 m | | | |
| C12 ^E | 3 | 384 | 209 | 110 | 21 h 58 m | 2 h 30 m | B | | Pt=20 mg/kg | Cn, M |
| C13 ^{E, S} | 2 | 413 | 63 | 393 | 17 h 54 m | 10 h 3 m | B | | | |
| C14 ^{E, S} | 3 | 640 | 305 | 126 | 16 h 48 m | 18 h 13 m | 1 h 25 m | 29 h 13 m | Pt=20 mg/kg | Pr, Mz, Py |
| C15 ^E | 3 | 958 | 190 | 303 | 27 h 28 m | 6 h 34 m | B | 9 h 44 m | Pt=5 mg/kg | Mz, Py |
| C16 ^E | 3 | 1002 | 201 | 299 | 20 h 35 m | 16 h 37 m | B | 26 h 47 m | Pt=40 mg/kg | M, D |

B, clinically treated before EEG commenced; C, neonates enrolled from the Cork University Maternity Hospital; Cn, clonazepam; D, intravenous diazepam; E, neonates with status epilepticus; M, morphine; Mz, midazolam; N, not given any anticonvulsant; Pr, paraldehyde; Pt, phenytoin; Py, pyridoxine; S, neonates who were already seizing at the time when EEG was commenced; Tr, trichloral hydrate.

phenobarbitone administration. In both hospitals, second-line anticonvulsant was either intravenous phenytoin or midazolam. Although standardized protocols for the use of anticonvulsants were similar in both hospitals, the choice of second-line anticonvulsant administration was at the discretion of the attending clinician.^{12 13} The timing and dose of each anticonvulsant as well as morphine administered were recorded in all neonates.

Throughout the study, EEG recording methods were identical at both hospitals. A Nicolet monitor (CareFusion NeuroCare, Wisconsin, USA) was used to record multichannel video-EEG, using the 10-20 system of electrode placement modified for neonates.¹⁴ EEG monitoring was commenced as soon as possible after birth and continued for at least 20 h of artefact-free EEG. Scalp electrodes were placed at F3, F4, C3, C4, T3, T4, O1, O2 and Cz locations to record the EEG activity from the frontal, central, temporal and occipital areas. Parietal electrodes (P3 and P4) were also used wherever possible. Impedances of below 5 k Ω were maintained. The entire EEG recording from each neonate was independently reviewed by two experienced encephalographers (GBB and SRM). Cases of disagreement were resolved by consensus. An electrographic seizure was defined as a sudden and evolving repetitive stereotyped waveform with a definite start, middle and end, lasting for at least 10 s¹⁵ on at least one EEG channel. Status epilepticus was defined as continuous¹⁶ or accumulative¹⁷ electrographic seizure activity lasting $\geq 50\%$ of each 1 h period. The recorded seizure burden was defined as the total duration of recorded electrographic seizures in minutes. Seizure number was counted as the number of seizure events recorded on the EEG. Mean seizure duration was calculated for all recorded electrographic seizures in each neonate.

STATISTICAL ANALYSIS

Inter-rater agreement between the two encephalographers was assessed using a Cohen's κ statistic. Continuous variables were described using medians and interquartile ranges (IQR) and categorical variables using frequencies. For comparisons between the two groups (non-cooled and cooled), the Mann-Whitney test was used for continuous variables and the χ^2 test or Fisher's exact test (in the case of small expected counts) was used for categorical variables. All statistical analyses were performed using PASW Statistics 17.0. All tests were two-sided and a p value <0.05 was considered to be statistically significant.

RESULTS

During the study, 107 neonates were diagnosed with HIE (figure 1). The clinical Sarnat grade for HIE was assigned as mild in 43, moderate in 34 and severe in 30 neonates. Among the 64 neonates with moderate or severe HIE, electrographic seizures were recorded in 37 neonates. Of these, six neonates were excluded from the study analysis. Four neonates with moderate HIE were excluded: two cooled neonates had secondary events shortly after EEG was commenced (one with cardiopulmonary arrest and the other with pulmonary haemorrhage), one cooled and one non-cooled neonate had less than 20 h of artefact-free EEG. Two neonates with severe HIE were excluded: one cooled neonate with a subsequent principal diagnosis of mitochondrial respiratory chain disease and one non-cooled neonate with less than 20 h of artefact-free EEG. The remaining 31 neonates formed our study group (16 non-cooled and 15 cooled). Tables 1–3 summarize the clinical characteristics of neonates in both groups.

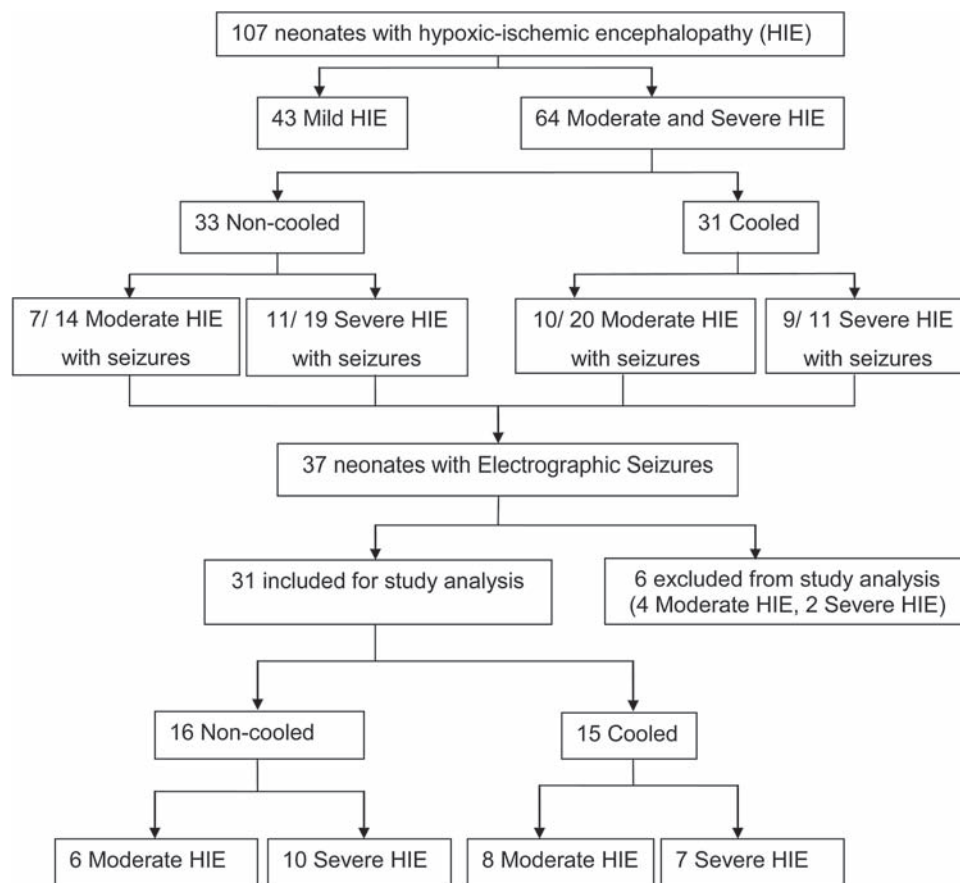


Figure 1 Flow diagram of study selection.

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Table 3 Individual characteristics of cooled neonates with hypoxia-ischaemic encephalopathy enrolled for this study

| Case | Clinical Sarnat | Recorded seizure burden (min) | Seizure number (n) | Mean seizure duration (s) | Age at first EEG seizure | Age at first-line anticonvulsant | Time from EEG seizure onset to treatment | Second-line anticonvulsant | | Other drugs | Cooling duration (age in h) |
|-----------------------|-----------------|-------------------------------|--------------------|---------------------------|--------------------------|----------------------------------|--|----------------------------|---------------|-------------|-----------------------------|
| | | | | | | | | Age | Total dose | | |
| L1 ^A | 2 | 19 | 4 | 283 | 10 h 59 m | 11 h 30 m | 31 m | | | M | 72 (12–84) |
| L2 ^A | 2 | 24 | 17 | 85 | 11 h 20 m | 12 h 25 m | 1 h 5 m | 26 h | Pt=20 mg/kg | M, Mz | 72 (8–90) |
| L3 ^E | 2 | 31 | 2 | 917 | 6 h 58 m | 7 h 25 m | 27 m | | | M | 72 (6–78) |
| C17 | 3 | 39 | 14 | 168 | 12 h 22 m | 6 h 10 m | B | | | M | 72 (0.25–72.25) |
| L4 | 2 | 48 | 12 | 241 | 24 h 23 m | 31 h 44 m | 7 h 21 m | | | M | 72 (6–78) |
| L5 ^{R, A} | 2 | 49 | 46 | 64 | 39 h 26 m | 24 h 23 m | B | | | M | 72 (9–81) |
| C18 ^E | 2 | 55 | 2 | 1658 | 8 h 17 m | 9 h 55 m | 1 h 38 m | | | M | 72 (2–74) |
| L6 ^W | 3 | 60 | 41 | 88 | 12 h | 12 h 51 m | 51 m | 20 h 11 m | Mz=150 mcg/kg | M | 33 (0.5–33.5) |
| C19 | 2 | 100 | 22 | 274 | 13 h 25 m | 15 h 38 m | 2 h 13 m | | | M | 72 (2–74) |
| C20 | 2 | 118 | 76 | 93 | 21 h 28 m | 7 h 20 m | B | | | M | 72 (3–75) |
| C21 ^{E, W} | 3 | 214 | 56 | 229 | 16 h 33 m | 17 h 3 m | 30 m | 28 h 33 m | Pt=20 mg/kg | M | 65 (0.8–66) |
| L7 ^{R, W} | 3 | 224 | 281 | 48 | 12 h 51 m | 21 h 22 m | 8 h 31 m | 25 h 52 m | Mz=340 mcg/kg | M | 23 (5–28) |
| C22 ^R | 3 | 244 | 185 | 79 | 42 h 13 m | 56 h 25 m | 14 h 12 m | 65 h 35 m | Pt=20 mg/kg | M | 72 (6–78) |
| L8 ^{E, R, W} | 3 | 289 | 161 | 108 | 13 h 5 m | 27 h 23 m | 14 h 18 m | | | M | 19 (5–24) |
| L9 ^{S, W} | 3 | 421 | 178 | 142 | 10 h 24 m | 13 h 35 m | 3 h 11 m | 13 h 55 m | Mz=330 mcg/kg | M | 66 (5–71) |

A, documented age onset of active cooling; passive cooling initiated earlier during transport; B, clinically treated before EEG commenced; C, neonates enrolled from Cork University Maternity Hospital; E, neonates with status epilepticus; L, neonates enrolled from University College London Hospital; M, morphine; Mz, midazolam; R, neonates with EEG seizures following discontinuation of cooling; Pt, phenytoin; S, neonates who were already seizing at the time when EEG record was commenced; W, shorter cooling period as part of withdrawal of life-sustaining support decision.

Eight of 16 non-cooled neonates and none of the cooled neonates received at least one dose of phenobarbitone before EEG monitoring commenced. However, there was no significant difference in the number of anticonvulsants received between the two groups (non-cooled: 2 (1–3) vs cooled: 1 (1–2); $p=0.274$) and in the total administered dose of first-line anticonvulsant (non-cooled: 30 (20–40) vs cooled: 20 (20–20) mg/kg; $p=0.203$). There was also no significant difference in the ages at which the first-line anticonvulsant (non-cooled: 12 (9–19) vs cooled: 14 (10–24) h; $p=0.504$) and the second-line anticonvulsant were administered (non-cooled: 28 (24–31) vs cooled: 26 (19–38) h; $p=0.556$). All cooled neonates received morphine compared with eight non-cooled neonates ($p=0.002$ from Fisher's exact test).

Cooling commenced at the median (IQR) age of 5 (2–6) h (table 3). In six of seven cooled neonates with severe HIE, cooling was commenced within 6 h of age. However, following decisions to withdraw life-sustaining support in five of these seven neonates, the duration of cooling and EEG monitoring were shorter. The recorded seizure burden in these neonates was higher than in cooled neonates with moderate HIE. In three of eight cooled neonates with moderate HIE, passive cooling commenced earlier during the transport to UCLH, but the recorded age at which active cooling commenced was after 6 h. Despite this, all eight neonates with moderate HIE received cooling for at least 72 h.

The inter-rater agreement for seizure identification was consistent with a high level of agreement ($\kappa=0.872$). In eight non-cooled and one cooled neonate, seizures were ongoing when EEG recording commenced. The postnatal age of first recorded electrographic seizure was similar in both groups (non-cooled: 18 (12–22) vs cooled: 13 (11–22) h; $p=0.252$). The median recorded seizure burden was significantly less in the cooled than in the non-cooled group (cooled: 60 (39–224) vs non-cooled 203 (141–406) min; $p=0.027$) (table 4). Between the cooled and the non-cooled group, there was no difference in the number of seizure events, mean seizure duration or the presence of status epilepticus ($p=0.105$, 0.192 and 0.095, respectively). An exploratory subgroup analysis was performed to assess the influence of cooling on neonates with different severity of encephalopathy

in non-cooled and cooled groups. Cooling had a significant reduction of recorded seizure burden in neonates with moderate HIE (non-cooled: 162 (97–262) vs cooled: 49 (26–89) min; $p=0.020$) while no such difference was seen in neonates with severe HIE (non-cooled: 223 (172–720) vs cooled: 224 (60–289) min; $p=0.558$).

Eleven cooled neonates had EEG monitoring after cooling was discontinued. Electrographic seizures were observed in 4 of 15 cooled neonates when cooling was discontinued (table 3). Two of the four cases had shorter duration of cooling when a decision was made to withdraw life-sustaining support (case L8 cooled for 19 h, case L7 cooled for 23 h). In the remaining two cases (cases C22 and L5), electrographic seizures were observed following discontinuation of cooling despite the fact that cooling started at 6 and 9 h, respectively after birth and continued for 72 h.

DISCUSSION

We have shown that term neonates with moderate HIE treated with whole-body cooling have a significantly lower electrographic seizure burden when compared with non-cooled neonates. This is the first study to quantify and compare the recorded seizure burden between non-cooled and cooled neonates using early and prolonged continuous multichannel video-EEG.

Previously published neonatal hypothermia trials could not accurately measure seizure burden as their protocols did not include multichannel EEG monitoring. These studies used clinical¹⁸ and/or aEEG monitoring^{7 19} for seizure recognition. The Neonatal Research Network Whole-Body Hypothermia Trial relied on the clinical recognition of seizures and when the authors adjusted their study for cooling and severity of encephalopathy, cooling did not appear to have any impact on the frequency of clinical seizures and outcome.¹⁸ However, clinical estimation of seizure burden is notoriously unreliable, with the majority of neonatal seizures being subclinical.^{4 20} The TOBY trial used aEEG as a recruitment and monitoring tool during cooling in some participating neonatal institutions.²¹ At recruitment, clinical seizures and seizures detected

Table 4 Characteristics of seizure burden in non-cooled and cooled groups

| All neonates | Non-cooled (n=16) | Cooled (n=15) | p Value |
|--|--------------------------|---------------------|----------------|
| Recorded seizure burden (min) | 203 (141–406) | 60 (39–224) | 0.027 |
| Total seizure numbers per neonate | 75 (42–180) | 41 (12–161) | 0.105 |
| Mean seizure duration (s) per neonate | 200 (101–324) | 142 (85–274) | 0.192 |
| Number of neonates with status epilepticus | 9 | 4 | 0.095* |
| Age onset of EEG (h) | 15 (5–20) | 6 (3–9) | 0.006 |
| Total EEG duration (h) | 55 (41–60) | 88 (75–101) | 0.001 |
| Moderate HIE | Non-cooled (n=6) | Cooled (n=8) | p Value |
| Recorded seizure burden (min) | 162 (97–262) | 49 (26–89) | 0.020 |
| Total seizure numbers per neonate | 42 (17–74) | 15 (3–40) | 0.174 |
| Mean seizure duration (s) per neonate | 362 (168–513) | 258 (87–759) | 0.519 |
| Severe HIE | Non-cooled (n=10) | Cooled (n=7) | p Value |
| Recorded seizure burden (min) | 223 (172–720) | 224 (60–289) | 0.558 |
| Total seizure numbers per neonate | 136 (56–203) | 161 (41–185) | 0.591 |
| Mean seizure duration (s) per neonate | 137 (98–293) | 108 (79–168) | 0.172 |

Data are median (IQR) or n.

* χ^2 test for the proportion of neonates with status epilepticus in non-cooled and cooled groups.

by aEEG were present in 67% (74/110) and 29% (33/115) of neonates, respectively and seizures were considered as a complication during cooling, with a decreasing incidence from days 1 to 4 (75–23%). Clinical recognition of seizures and the aEEG is known to underestimate the true seizure burden. The aEEG cannot detect short seizures, seizures which do not generalize and low voltage seizures.⁶

In fetal sheep, cooling was associated with a marked reduction in the amplitude of seizures and epileptiform activities in the first 6 h after a complete umbilical cord occlusion.²² The duration of individual electrographic seizures was reduced in the cooled compared to the non-cooled asphyxiated piglets.²³ Hypothermia to 30 or 33°C has been shown to completely inhibit the release of glutamate in a rat model of cerebral ischaemia.²⁴ Other effects of cooling such as reduced cytotoxic oedema by reducing amino acid release²⁵ and inhibition of free oxygen radicals²⁶ may have an impact on the reduction in seizure burden.

The results of exploratory analysis showed that the recorded seizure burden was only significantly reduced in cooled neonates with moderate HIE. Possibly, this is related to the higher recorded seizure burden in five of seven cooled neonates with severe HIE who had shorter durations of cooling and EEG monitoring following decisions to withdraw life-sustaining support. Interestingly, the analysis of three neonatal hypothermia trials has revealed that the primary outcome of death and disability at 18 months was significantly reduced by cooling neonates with moderate but not severe HIE.⁷ However, Simbruner *et al.* has shown that cooling was strongly neuroprotective even in severe HIE.¹⁹ Therefore, it is important to emphasize that further data are required to clarify whether cooling is appropriate for severe HIE, before clinical decisions are made to abort cooling neonates with severe HIE.

This study has a retrospective design which may have led to some bias. Both cohorts were selected as they were encephalopathic and at high risk of developing seizures. Both groups had continuous multichannel EEG monitoring, and the standard protocol for monitoring was the same at both time points and in both hospitals. We did anticipate a potential bias relating to the choice of anticonvulsants used, as administration was at the discretion of different attending clinicians in both hospitals at that point in time. To date, there is still no consensus on a standard protocol for the use of anticonvulsants among neonatologists.^{12 13} The recorded seizure burden

remained higher in non-cooled neonates, despite the fact that they received anticonvulsants earlier. However, there was no significant difference between the groups in the number, dose and age when the first- and second-line anticonvulsants were administered. One of the strengths of this study is that the same reviewers analyzed the EEG recordings of both cohorts using a standardized grading system. EEGs were also recorded in both groups on the same equipment. As we were using a historical cohort, over time some increase in the ability of the EEG annotators to recognize seizures through increasing use of early continuous EEG may have occurred. However, this should have increased the seizure burden in the more recent cooled group. This bias only serves to strengthen our findings.

Phenobarbitone remains the most commonly used first-line anticonvulsant in neonatal units worldwide.^{12 13} It has been shown to augment the neuroprotective effect of hypothermia.²⁷ However, phenobarbitone has been shown to be ineffective in controlling electrographic seizures in non-cooled neonates;^{28 29} this reduced efficacy has been linked to the altered neuronal chloride transport in the developing brain.³⁰ In our study, plasma phenobarbitone levels were not routinely measured. It is known that the half-life of phenobarbitone is significantly increased in cooled neonates and plasma drug levels will accumulate due to reduced hepatic metabolism during hypothermia.^{31 32} Sedative and anaesthetic medications have been shown to facilitate the therapeutic effects of cooling.²³ All cooled neonates and half of non-cooled neonates in our study received morphine. However, morphine does not possess anticonvulsive properties and therefore cannot explain the measured difference in the recorded seizure burden between the two groups.

There was no significant difference in Apgar scores between both groups, although the pH was significantly lower in the cooled group. This may reflect more severe disease in the cooled group. However, we have previously shown that neither the condition at birth nor the degree of metabolic acidosis reliably predicts electrographic neonatal seizures³³ and therefore we do not think that this had any influence on the seizure burden in the cooled group. All non-cooled neonates would have qualified for cooling if it was available at the time of recruitment. The time of onset and the duration of EEG recording between non-cooled and cooled groups were significantly different. Several non-cooled neonates were already

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experiencing seizures when EEG recording commenced; the recorded seizure burden in this group may have been underestimated. Despite this, and the fact that there was a longer EEG recording time which increased the possibility of capturing more seizures in the cooled group, the overall recorded seizure burden was still lower in the cooled group.

Two recent hypothermia studies have not quantified the recorded seizure burden and a control cohort was not made available for comparison.^{34 35} In another study, seizures occurred less frequently in the cooled group but this was not significantly different with a control cohort.³⁶ It would no longer be ethical to randomize HIE neonates to normothermia. For this reason, although our study sample size is small, our non-cooled HIE cohort with prolonged continuous EEG monitoring is unlikely to be replicated.

In summary, we found that cooling was associated with a decreased electrographic seizure burden in neonates with HIE. A reduced seizure burden may lead to a reduction in neuronal damage, and may help explain the observed improvement in long-term neurodevelopmental outcome in cooled neonates with moderate HIE. Further studies using prolonged continuous multichannel EEG monitoring are undoubtedly indicated.

Correction notice This article has been corrected since it was published Online First. The word electroencephalographers has been corrected to encephalographers and "prefix cases" has been deleted from the list of abbreviations in table 3.

Contributors GB, DM and JR developed the hypothesis and conceived the study. EL, DM, IK, GB, SM compiled and annotated the EEG data. GB and JR obtained funding for the study. EL, GB, SM, DM and NS analyzed and interpreted the EEGs. Statistical analysis was conducted by VL.

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Competing interests None.

Patient consent Obtained.

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REFERENCES

- Volpe JJ. *Neurology of the Newborn*. Fifth edition. Philadelphia, PA: WB Saunders Company 2008:203–44.
- Glass HC, Glidden D, Jeremy RJ, *et al*. Clinical Neonatal Seizures are independently associated with outcome in infants at risk for hypoxic-ischemic brain injury. *J Pediatr* 2009;**155**:318–23.
- van Rooij LG, de Vries LS, Handryastuti S, *et al*. Neurodevelopmental outcome in term infants with status epilepticus detected with amplitude-integrated electroencephalography. *Pediatrics* 2007;**120**:e354–63.
- Murray DM, Boylan GB, Ali I, *et al*. Defining the gap between electrographic seizure burden, clinical expression and staff recognition of neonatal seizures. *Arch Dis Child Fetal Neonatal Ed* 2008;**93**:F187–91.
- Shellhaas RA, Soaita AI, Clancy RR. Sensitivity of amplitude-integrated electroencephalography for neonatal seizure detection. *Pediatrics* 2007;**120**:770–7.
- Rennie JM, Chorley G, Boylan GB, *et al*. Non-expert use of the cerebral function monitor for neonatal seizure detection. *Arch Dis Child Fetal Neonatal Ed* 2004;**89**:F37–40.
- Edwards AD, Brocklehurst P, Gunn AJ, *et al*. Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data. *BMJ* 2010;**340**:c363.
- National Institute for Health and Clinical Excellence (NICE). Controlled cooling to treat newborn babies with brain injury caused by oxygen shortage during birth. Information about NICE interventional procedure guidance **347**; Reference No N2184. www.nice.org.uk (accessed 10 May 2010).
- Thoresen M, Penrice J, Lorek A, *et al*. Mild hypothermia after severe transient hypoxia-ischemia ameliorates delayed cerebral energy failure in the newborn piglet. *Pediatr Res* 1995;**37**:667–70.
- Gunn AJ, Gunn TR, de Haan HH, *et al*. Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal lambs. *J Clin Invest* 1997;**99**:248–56.
- Evans DJ, Levene M. Hypoxic-ischemic injury. In: Rennie JM, Robertson NRC, eds. *Textbook of Neonatology*. Edinburgh: Churchill Livingstone 1999:1231–51.
- Vento M, de Vries LS, Alberola A, *et al*. Approach to seizures in the neonatal period: a European perspective. *Acta Paediatr* 2010;**99**:497–501.
- Bartha AI, Shen J, Katz KH, *et al*. Neonatal seizures: multicenter variability in current treatment practices. *Pediatr Neurol* 2007;**37**:85–90.
- Klem GH, Lüders HO, Jasper HH, *et al*. The ten-twenty electrode system of the International Federation. The International Federation of Clinical Neurophysiology. *Electroencephalogr Clin Neurophysiol Suppl* 1999;**52**:3–6.
- Clancy RR, Legido A. The exact ictal and interictal duration of electroencephalographic neonatal seizures. *Epilepsia* 1987;**28**:537–41.
- Scher MS, Hamid MY, Steppe DA, *et al*. Ictal and interictal electrographic seizure durations in preterm and term neonates. *Epilepsia* 1993;**34**:284–8.
- Ortibus EL, Sum JM, Hahn JS. Predictive value of EEG for outcome and epilepsy following neonatal seizures. *Electroencephalogr Clin Neurophysiol* 1996;**98**:175–85.
- Kwon JM, Guillet R, Shankaran S, *et al*. Clinical seizures in neonatal hypoxic-ischemic encephalopathy have no independent impact on neurodevelopmental outcome: secondary analyses of data from the neonatal research network hypothermia trial. *J Child Neurol* 2011;**26**:322–8.
- Simbruner G, Mittal RA, Rohmann F, *et al*. Systemic hypothermia after neonatal encephalopathy: outcomes of neo.nEURO.network RCT. *Pediatrics* 2010;**126**:e771–8.
- Yap V, Engel M, Takenouchi T, *et al*. Seizures are common in term infants undergoing head cooling. *Pediatr Neurol* 2009;**41**:327–31.
- Azzopardi D, Strohm B, Edwards AD, *et al*. Treatment of asphyxiated newborns with moderate hypothermia in routine clinical practice: how cooling is managed in the UK outside a clinical trial. *Arch Dis Child Fetal Neonatal Ed* 2009;**94**:F260–4.
- Bennet L, Dean JM, Wassink G, *et al*. Differential effects of hypothermia on early and late epileptiform events after severe hypoxia in preterm fetal sheep. *J Neurophysiol* 2007;**97**:572–8.
- Tooley JR, Satas S, Porter H, *et al*. Head cooling with mild systemic hypothermia in anesthetized piglets is neuroprotective. *Ann Neurol* 2003;**53**:65–72.
- Busto R, Globus MY, Dietrich WD, *et al*. Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain. *Stroke* 1989;**20**:904–10.
- Nakashima K, Todd MM. Effects of hypothermia on the rate of excitatory amino acid release after ischemic depolarization. *Stroke* 1996;**27**:913–18.
- Globus MY, Alonso O, Dietrich WD, *et al*. Glutamate release and free radical production following brain injury: effects of posttraumatic hypothermia. *J Neurochem* 1995;**65**:1704–11.
- Barks JD, Liu YQ, Shangquan Y, *et al*. Phenobarbital augments hypothermic neuroprotection. *Pediatr Res* 2010;**67**:532–7.
- Painter MJ, Scher MS, Stein AD, *et al*. Phenobarbital compared with phenytoin for the treatment of neonatal seizures. *N Engl J Med* 1999;**341**:485–9.
- Sarkar S, Barks JD, Bapuraj JR, *et al*. Does phenobarbital improve the effectiveness of therapeutic hypothermia in infants with hypoxic-ischemic encephalopathy? *J Perinatal* Published Online First: 28 April 2011. doi:10.1038/jp.2011.41.
- Dzhala VI, Talos DM, Sdrulla DA, *et al*. NKCC1 transporter facilitates seizures in the developing brain. *Nat Med* 2005;**11**:1205–13.
- Róka A, Melinda KT, Vársárhelyi B, *et al*. Elevated morphine concentrations in neonates treated with morphine and prolonged hypothermia for hypoxic ischemic encephalopathy. *Pediatrics* 2008;**121**:e844–9.
- Filippi L, la Marca G, Cavallaro G, *et al*. Phenobarbital for neonatal seizures in hypoxic ischemic encephalopathy: a pharmacokinetic study during whole body hypothermia. *Epilepsia* 2011;**52**:794–801.
- Murray DM, Ryan CA, Boylan GB, *et al*. Prediction of seizures in asphyxiated neonates: correlation with continuous video-electroencephalographic monitoring. *Pediatrics* 2006;**118**:41–6.
- Wusthoff CJ, Dlugos DJ, Gutierrez-Colina A, *et al*. Electrographic seizures during therapeutic hypothermia for neonatal hypoxic-ischemic encephalopathy. *J Child Neurol* 2011;**26**:724–8.
- Nash KB, Bonifacio SL, Glass HC, *et al*. Video-EEG monitoring in newborns with hypoxic-ischemic encephalopathy treated with hypothermia. *Neurology* 2011;**76**:556–62.
- Hamelin S, Delnard N, Cneude F, *et al*. Influence of hypothermia on the prognostic value of early EEG in full-term neonates with hypoxic ischemic encephalopathy. *Neurophysiol Clin* 2011;**41**:19–27.



Cooling and seizure burden in term neonates: an observational study

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Early Postnatal EEG Features of Perinatal Arterial Ischaemic Stroke with Seizures

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Abstract

Background: Stroke is the second most common cause of seizures in term neonates and is associated with abnormal long-term neurodevelopmental outcome in some cases.

Objective: To aid diagnosis earlier in the postnatal period, our aim was to describe the characteristic EEG patterns in term neonates with perinatal arterial ischaemic stroke (PAIS) seizures.

Design: Retrospective observational study.

Patients: Neonates >37 weeks born between 2003 and 2011 in two hospitals.

Method: Continuous multichannel video-EEG was used to analyze the background patterns and characteristics of seizures. Each EEG was assessed for continuity, symmetry, characteristic features and sleep cycling; morphology of electrographic seizures was also examined. Each seizure was categorized as electrographic-only or electroclinical; the percentage of seizure events for each seizure type was also summarized.

Results: Nine neonates with PAIS seizures and EEG monitoring were identified. While EEG continuity was present in all cases, the background pattern showed suppression over the infarcted side; this was quite marked (>50% amplitude reduction) when the lesion was large. Characteristic unilateral bursts of theta activity with sharp or spike waves intermixed were seen in all cases. Sleep cycling was generally present but was more disturbed over the infarcted side. Seizures demonstrated a characteristic pattern; focal sharp waves/spike-polyspikes were seen at frequency of 1–2 Hz and phase reversal over the central region was common. Electrographic-only seizure events were more frequent compared to electroclinical seizure events (78 vs 22%).

Conclusions: Focal electrographic and electroclinical seizures with ipsilateral suppression of the background activity and focal sharp waves are strong indicators of PAIS. Approximately 80% of seizure events were the result of clinically unsuspected seizures in neonates with PAIS. Prolonged and continuous multichannel video-EEG monitoring is advocated for adequate seizure surveillance.

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Introduction

Perinatal arterial ischaemic stroke (PAIS) occurs approximately 1 in 2500 livebirths and is recognized as a common cause of early onset neonatal seizures. [1] Approximately 20% of neonatal seizures are due to PAIS, [2] and neonatal seizures have been noted in up to 26% of neonates with PAIS. [3] Generally, neonates with PAIS are non-encephalopathic but those with significant seizure burden can be neurologically abnormal, making the distinction from seizures due to other causes such as hypoxia-ischaemia difficult in the acute neonatal period. [4] The diagnosis

of PAIS should be suspected when seizures are observed in non-encephalopathic neonates within the first 48 hours of birth. [5] While cranial ultrasound scans have been shown to have good diagnostic capabilities when performed after day 4, [6] confirmation of diagnosis is only reliably achieved with magnetic resonance imaging (MRI); however this facility is not readily available in many institutions.

Electroencephalogram (EEG) or amplitude integrated-EEG (aEEG) is now one of the first diagnostic tools available at the bedside in the neonatal intensive care unit for the assessment of

cerebral function. Most studies in PAIS have described EEG changes in the first week after birth, but typical changes observed in the first 48 hours after birth have not been described. Early EEG may distinguish neonates with PAIS from those with hypoxic-ischaemic encephalopathy (HIE) [3] and other aetiologies, providing invaluable support for clinical decision-making and counselling. The aEEG has been used to obtain additional information in neonates with PAIS by van Rooij *et al.* [7] and Mercuri *et al.* [8]; however these studies have not given details on the characteristics of electrographic seizures. Early accurate recognition of PAIS would be helpful in distinguishing neonates with seizures who do not fulfil the current criteria for therapeutic hypothermia, but who require thrombophilic screening and high quality MRI for diagnosis and prognosis. The aim of our study was to characterize the early postnatal EEG findings in term neonates with PAIS who had seizures.

Methods

Ethics statement

This study was approved by the Clinical Research Ethics Committees of the Cork Teaching hospitals, Ireland and the National Health Service in the United Kingdom (UK), via the Integrated Research Application Service. Written, informed consent was obtained from at least one parent of each neonate who participated in this study.

Patients

Neonates were enrolled from Cork University Maternity Hospital (CUMH), Ireland between June 2003 and October 2011 and University College London Hospital (UCLH), UK from January 2009 to October 2011 as part of an ongoing study of neonatal seizures. Neonates >37 weeks gestation were enrolled for EEG monitoring if they fulfilled at least one of the following criteria: Apgar score <6 at five minutes; a continued need for resuscitation after birth; any clinical evidence of encephalopathy or seizures within 72 hours of age. The diagnosis of PAIS was based on neuroimaging evidence of focal infarction affecting at most two arterial territories. Study analysis included only neonates with PAIS who had electrographic seizures. Neonates with HIE, infections, inborn errors of metabolism, blood disorders, venous or multiple infarctions were excluded due to differing pathogenesis and clinical manifestations when compared to those with focal arterial infarction.

Clinical features

All clinical seizures were treated as well as seizures recognized by the clinical team interpreting the aEEG. The aEEG used to confirm suspected seizures was also used as an aid in clinical decision-making at the bedside. Concern regarding any abnormal behaviour or aEEG pattern prompted a review of the multichannel EEG from the neurophysiologist in each hospital. Immediate reporting of the multichannel EEG was not always available; the aEEG and clinical suspicion were the mainstays of seizure diagnosis. Phenobarbitone was the first-line anticonvulsant administered to a maximum dose of 40 mg/kg intravenously. Second-line anticonvulsants were administered if clinical and/or electrographic seizures recurred following phenobarbitone administration. In both hospitals, second-line anticonvulsant was either intravenous phenytoin or midazolam. Although standardized protocols for the use of anticonvulsants were similar in both hospitals, the choice of second-line anticonvulsant administration was at the discretion of the attending neonatologist. The timing

and dose of each anticonvulsant as well as morphine administered were recorded in all neonates.

EEG features

Clinical details of all neonates were obtained at the time of monitoring. Throughout the study, EEG recording methods were identical at both hospitals. A Nicolet monitor (Carefusion NeuroCare, Wisconsin, USA) was used to record multichannel video-EEG, using the 10–20 system of electrode placement modified for neonates. [9] EEG monitoring was commenced when recruitment criteria were met and continued for at least 20 hours. Scalp electrodes were placed at F3, F4, C3, C4, T3, T4, O1, O2 and Cz locations to record EEG activity from the frontal, central, temporal and occipital areas. Parietal electrodes (P3–P4) were also used where possible. Impedances below five k Ω were maintained. Simultaneous bilateral aEEG trends, electrocardiogram and respiration traces were also displayed on the monitor.

All EEG recordings from each neonate were independently reviewed by an experienced neonatal electroencephalographer (GBB). The entire background EEG pattern was graded and assessed for continuity, symmetry, synchrony and other specific features. Sleep cycling was assessed as being present, absent or disturbed in each neonate; a disturbed sleep cycling signified an interruption to the expected sleep cycle architecture of healthy term neonates. [10] Significant EEG suppression was defined as EEG activity below five μ V in all EEG channels for at least 10 seconds respectively. The morphology of seizures was also assessed. An electrographic seizure was defined as a sudden and evolving repetitive stereotyped waveform with a definite start, middle and end, lasting for at least 10 seconds [11] on at least one EEG channel. Status epilepticus was defined as continuous or accumulative electrographic seizure activity lasting $\geq 50\%$ of a one-hour period. [12]

Any associated clinical correlates with all electrographic seizures annotated were analyzed using the simultaneous video recording. Electrographic-only seizures were defined as clear electrographic seizures without any clinical correlates. [13] Electroclinical seizures were defined as electrographic seizures accompanied with behavioural correlates. Clinical seizures were defined as paroxysmal alterations in neurological function (behaviour, motor or autonomic); the description was based on those categorized by Volpe. [2] Subtle seizures were defined as paroxysmal behaviours (including changes in autonomic parameters) which were not clearly clonic, tonic or myoclonic seizures [2] and included behaviours such as eye blinking, pedalling or cycling movements of the limbs, hiccups, sucking or chewing movements and apnoeic spells.

Radiographic features

MRI studies were performed in a Siemens Avanto 1.5 Tesla unit (Siemens Ag, Erlangen, Germany) and CT scanning was performed using a Toshiba Aquilion 4-detector row CT (Toshiba, Tochigi-ken, Japan). All imaging studies were performed without sedation. Neonates were transferred to the MRI scanner in an MRI-compatible incubator with integrated neonatal array coils (MR Diagnostics Incubator, Lammers Medical Technology GmbH, Luebeck, Germany). The arterial territory and estimated size of cerebral infarction based on methods described by Marks *et al.*, [14] were reported by an experienced paediatric radiologist (COB).

Statistical analysis

The total seizure burden was defined as the total duration of recorded electrographic seizures in minutes. Electrographic

seizure window was defined as the timepoint between the first and last recorded electrographic seizure in hours. Seizure burden was also expressed in terms of seizure per hour and was calculated using a formula:

Seizure burden = total seizure burden (minutes)/ electrographic seizure window (hours).

In each neonate, the mean seizure duration is calculated as the proportion of the total seizure burden in seconds relative to the number of seizures.

Mean seizure duration = total seizure burden (in seconds) / total number of seizures.

To avoid neonates with many seizures having much influence on the results, summary measures were calculated for each neonate. These summary measures were percentages of the number of seizure events and the seizure burden (seizure duration in minutes) associated with electrographic-only, electroclinical seizures and the duration when viewing of the video was obscured (for example during a medical procedure); they were calculated relative to the total number of electrographic seizures and the total seizure burden (seizure duration in minutes). For example:

% number of electrographic-only seizures = (the number of electrographic-only seizures/ the total number of seizures) * 100

% seizure burden of electrographic-only seizures = (the seizure burden of electrographic-only seizures/ the total seizure burden) * 100

% number of electroclinical seizures = (the number of electroclinical seizures/ the total number of seizures) * 100

% seizure burden of electroclinical seizures = (the seizure burden of electroclinical seizures/ the total seizure burden) * 100.

These summary measures were then described across all neonates using medians and interquartile ranges (IQR). For paired comparisons, the Wilcoxon signed-rank test was used. All statistical analyses were performed using SPSS Statistics 20.0 (IBM SPSS Statistics, Illinois, USA). All tests were two-sided; p -value <0.05 was considered to be statistically significant.

Results

During the study, nine neonates with PAIS who had continuous early EEG monitoring had electrographic seizures. Five neonates had coagulation testing and none had thrombophilic disorders. Table 1 lists the clinical demographics and outlines the MRI findings in eight of the nine neonates with various degrees of middle cerebral artery (MCA) infarction; one neonate had CT imaging. Cranial imaging was undertaken at a median (IQR) of 5 (3–12) days after birth.

Table 2 summarizes the background EEG and seizure characteristics for each neonate. In all neonates, a continuous background pattern was present but voltage suppression and intermittent sharp theta discharges were seen over the infarcted side (figure 1). Background EEG suppression was greatest in cases where the estimated size of infarction was larger than 66% of one hemisphere. Sleep cycling was present in all cases but disturbed in 5 of the 9 neonates. The morphology of seizures in neonates with PAIS showed a characteristic pattern in all cases (figure 2). Spike and polyspike waves at a frequency of 1–2 Hz were seen over the infarcted side and phase reversal of these spikes over the central region was evident as the seizure evolved. Higher frequency temporal discharges were seen during apnoea in a neonate who presented with dusky episodes.

Of 536 electrographic seizures identified from multichannel EEG in this cohort of neonates with PAIS; 519 were classified (table 2). Accumulatively, there were more electrographic-only seizure events ($n = 405$; 78%) than electroclinical seizure events

($n = 114$; 22%). Summary measures of each neonate showed that the median (IQR) electrographic-only seizure events was higher than electroclinical seizure events [66 (52–90) *vs* 29 (8–40)%; $p = 0.051$]. Subtle seizures were noted in six of nine neonates and manifested activities such as pedalling or cycling movements of the limbs, sucking or chewing movements. Other occasional subtle seizures noted were hiccups and eye blinking episodes. When electroclinical seizures were subdivided, there were more subtle ($n = 61$; 12%) than clonic seizures ($n = 53$; 10%) [median (IQR) of subtle *vs* clonic seizures = 12 (0–22) *vs* 7 (2–24)%; $p = 0.553$]. The median percentage of seizure burden of electrographic-only was higher than electroclinical seizures [49 (31–88) *vs* 44 (12–51)%; $p = 0.515$]. This is despite the significantly shorter median duration of electrographic-only when compared to electroclinical seizures [100 (55–173) *vs* 181 (95–359) seconds; $p < 0.001$].

The temporal distribution of electrographic-only and electroclinical seizures with anticonvulsant administration superimposed for each neonate are shown in figure 3. In four of nine neonates (cases 1, 2, 3 and 6), anticonvulsants were administered prior to prolonged multichannel EEG monitoring, hence before the first electrographic seizure. All nine neonates with PAIS received first-line anticonvulsants at 34 (20–46) hours while seven neonates received second-line anticonvulsants at 48 (29–66) hours.

Discussion

The background EEG generally showed suppression over the affected side; this was quite marked ($>50\%$ amplitude reduction) if the infarction was large. Characteristic unilateral theta bursts with intermixed sharp or spike waves were seen in all cases over the infarcted side. Sleep cycling was generally present but was more disturbed over the infarcted side. Seizures in neonates with PAIS appear to have a characteristic pattern and in all cases, focal sharp waves/spike-polyspike seizure discharges were seen at a frequency of 1–2 Hz over the area of infarction. In our experience, the morphology of these seizures is quite characteristic and markedly different from seizures due to HIE. [15] All neonates in our series had MCA involvement; seizures were generally seen over the central region and phase reversal of spike and polyspike discharges were a common finding. This is the first study to describe these characteristic EEG findings in a series of neonates with PAIS in the early postnatal period; these findings may prove very useful for early diagnosis of neonates with seizures.

Indeed PAIS tends to be a clinical diagnosis when three important findings are present: no clear history of HIE, seizure onset beyond 12 hours after birth and focal seizures. In many instances when the affected cases are discussed retrospectively, subtle details are often missed; they usually revealed a slightly complicated antenatal history such as mild changes on the cardiotocogram or meconium stained delivery. [16] Apgar scores and clinical history may be subjective. We advocate the use of the EEG as an adjunct to suggest the early diagnosis of PAIS during the neonatal period when clinical suspicions are aroused.

Comparing one-channel with the two-channel aEEG recordings in 34 neonates who had seizures due to unilateral brain injury, van Rooij *et al.* showed more varied seizures patterns, asymmetry in the background activity and a difference in sleep cycling on the ipsilateral side, [7] however this study gave no specific analysis on a subgroup of neonates who had PAIS ($n = 5$) or specifically those who had MCA involvement ($n = 3$). Using a four-channel aEEG in 19 neonates with PAIS (6 neonates with asymmetrical and 2 with bilateral sharp waves/ spikes, 8 no seizures, 3 not recorded), Mercuri *et al.* showed that the presence of seizures accompanied by a normal background EEG was not related to abnormal

Table 1. Demographics and neuroimaging features of neonates in the order of increasing seizure burden.

| Neonate | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|------|-----------------------------|-------|------------|---------------------|----------|-------|------------|-------|
| Birthweight (grams) | 3700 | 3740 | 3750 | 3410 | 2830 | 3420 | 3160 | 3670 | 3480 |
| Gestation (weeks) | 40 | 39 | 41 | 41 | 39 | 41 | 39 | 41 | 41 |
| Gender | Male | Male | Male | Female | Female | Female | Male | Male | Male |
| Perinatal events | None | Polyhydramnios, PROM (36 h) | NRCTG | None | NRCTG, PROM (>18 h) | None | NRCTG | FTP | FTP |
| Mode of delivery | VV | VV | EMCS | Forceps | EMCS | Ventouse | EMCS | EMCS | EMCS |
| First pH | 7.42 | 7.04 | 7.13 | 7.29 | 7.00 | 7.34 | 7.30 | 7.41 | 7.27 |
| 5 min Apgar | 10 | 9 | 9 | 10 | 6 | 7 | 10 | 10 | 10 |
| Age at first clinical seizure (hours) | 36 | 54 | 20 | 6 | 47 | 33 | 15 | 18 | 33 |
| First clinical seizure | RUL | Dusky episodes | LS | RS | LLL | RS | RUL | RUL | LUL |
| Age at EEG (hours) | 54 | 59 | 26 | 9 | 53 | 3 | 18 | 19 | 36 |
| Age at first recorded EEG seizure (hours) | 54 | 60 | 26 | 9 | 53 | 39 | 19 | 19 | 36 |
| EEG duration (hours) | 25 | 70 | 49 | 39 | 44 | 46 | 49 | 63 | 229 |
| Cerebral infarction | LMCA | LMCA | RMCA | LMCA, RMCA | RMCA | LMCA | LMCA | LMCA, LPCA | RMCA |
| Age at cranial imaging (days) | 5 | 8 | 29 | 3 | 10 | 3 | 2 | 3 | 14 |
| Estimated size of infarction (%) | <33 | >66 | <33 | <33 | 33–66 | 33–66 | <33 | >66 | 33–66 |

EMCS, emergency Caesarean section; FTP, failure to progress; LLL, left lower limb clonic; LMCA, left middle cerebral artery; LPCA, left posterior cerebral artery; LS, left-sided clonic; LUL, left upper limb clonic movements; NRCTG, non-reassuring cardiotocogram; PROM, premature rupture of membranes; RMCA, right middle cerebral artery; RS, right-sided clonic; RUL, right upper limb clonic; VV, vertex vaginal.

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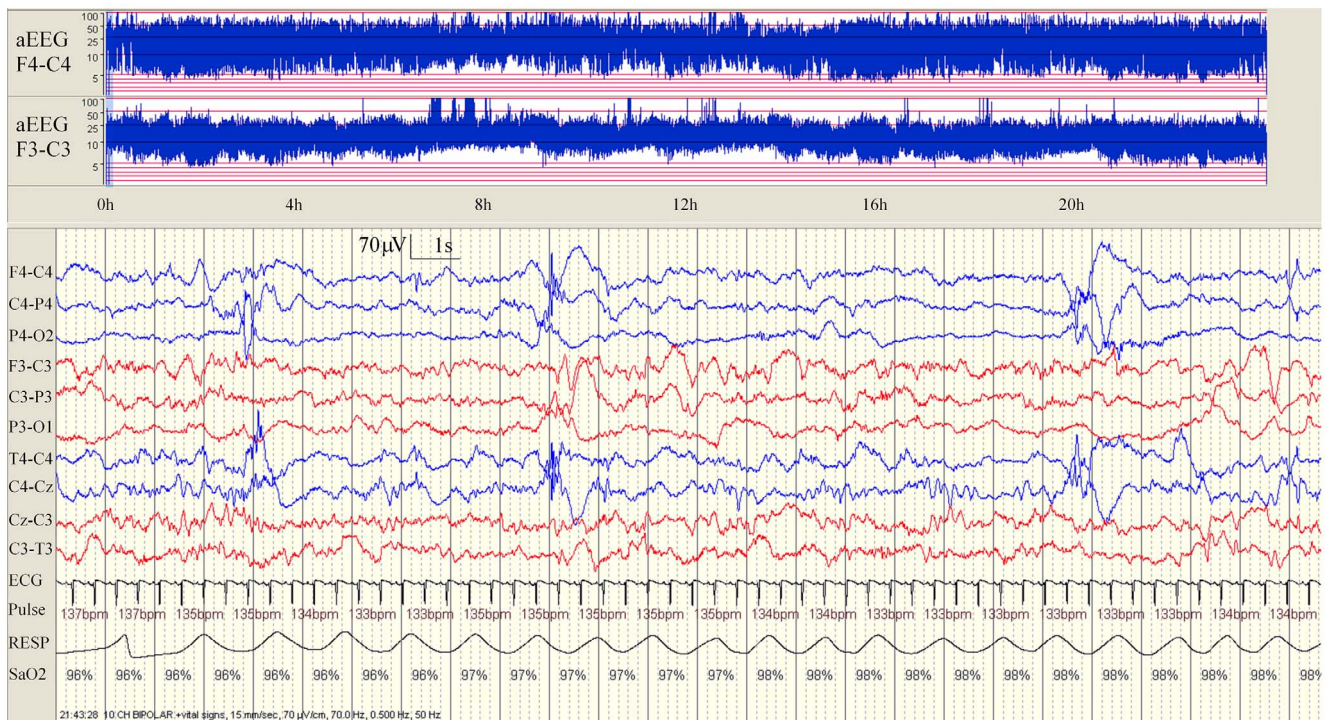


Figure 1. Background EEG pattern in a neonate (case 9) with a right middle cerebral artery infarction. Note the mild voltage reduction over the right hemisphere on EEG (blue channels) which is also evident on the aEEG with a wider band on the right in comparison to the left side. In addition, intermittent right-sided bursts of higher voltage sharpened theta activity are also evident. Some sleep cycling is also present over the left albeit disturbed but this is absent over the right side.
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outcome; [8] this indicates that both factors are poor predictors of outcome. Although our study was not aimed to assess outcome, we believe that an abnormal background and the presences of seizures have a much higher prognostic value. Also, the study by Mercuri *et al.* had not assessed seizures as an independent factor in determining outcome. [8] Multichannel EEG has been shown to be more accurate than the aEEG in detecting seizures. Our EEG findings based on multichannel EEG recordings are similar to studies by van Rooij *et al.* [7] and Mercuri *et al.* [8] which used the aEEG, however we have provided more details on the characteristics of seizures early in the neonatal period in terms of seizure morphology and more detailed seizure characteristics in a cohort of neonates with PAIS.

Several studies have reported the electrographic seizure burden in neonates with HIE, [17], [18] but none has quantified seizure burden in neonates with PAIS using continuous multichannel EEG. A study by Rafay *et al.* compared the EEG characteristics between neonates with PAIS and HIE; [3] they showed that there was no significant difference in the number of neonates who had electrographic seizures (PAIS *vs* HIE: 7/27 *vs* 13/35; $p = 0.350$). Although their study contributed further to our understanding of neonatal seizures, the results were limited because EEG findings were described exclusively from EEG reports generated by a neurophysiology service. In our study, we have explored further on the multichannel EEG recordings. The overall seizure burden was high in our study; prolonged multichannel video-EEG monitoring showed that the number of seizures is higher than clinically apparent. In our study, anticonvulsants were administered when there was a clinical concern of seizures. The use of anticonvulsants may have resulted in more electrographic-only seizures [19]; and in our study we have shown that 80% of seizure events were

electrographic-only seizures. The high number of seizures which we uncovered in this group of neonates was surprising but reinforces the need for early and continuous EEG monitoring in this group of neonates. In comparison, electroclinical dissociation has been reported to occur up to 28% of neonates with HIE; however this figure was based on aEEG findings in neonates above 32 weeks gestation and its association with anticonvulsant administration was not described. [20] The studies by van Rooij *et al.* [7] and Mercuri *et al.* [8] did not provide information on the dissociation of seizures. Many of the previous studies reported the clinical response to anticonvulsants without any EEG monitoring. [21–23] It is known that anticonvulsants can be a sedative agent and lead to electroclinical uncoupling or dissociation. [24] Clinical seizures are therefore a poor indicator when it comes to assessing the response to anticonvulsants; hence the true response of anticonvulsants in seizure control in neonates with PAIS remains unknown. Our study highlights that despite the use of anticonvulsants, under tight EEG monitoring, there are still ongoing electrographic seizures in neonates with PAIS. Neonatologists should be aware of this when treating neonates with PAIS who are already treated with initial anticonvulsants, particularly in the absence of EEG monitoring. This also explains why several neonates in our study had many hours of repetitive seizures and were not treated with anticonvulsants. We believe that this study is the first to demonstrate the high seizure burden in PAIS using continuous multichannel EEG monitoring and is thus of significant and practical clinical importance.

The MCA is the most commonly involved artery for ischaemic infarction in term neonates (the posterior branch irrigates the occipital, temporal and posterior parietal areas, while the anterior branch irrigates the prefrontal, precentral, central and anterior

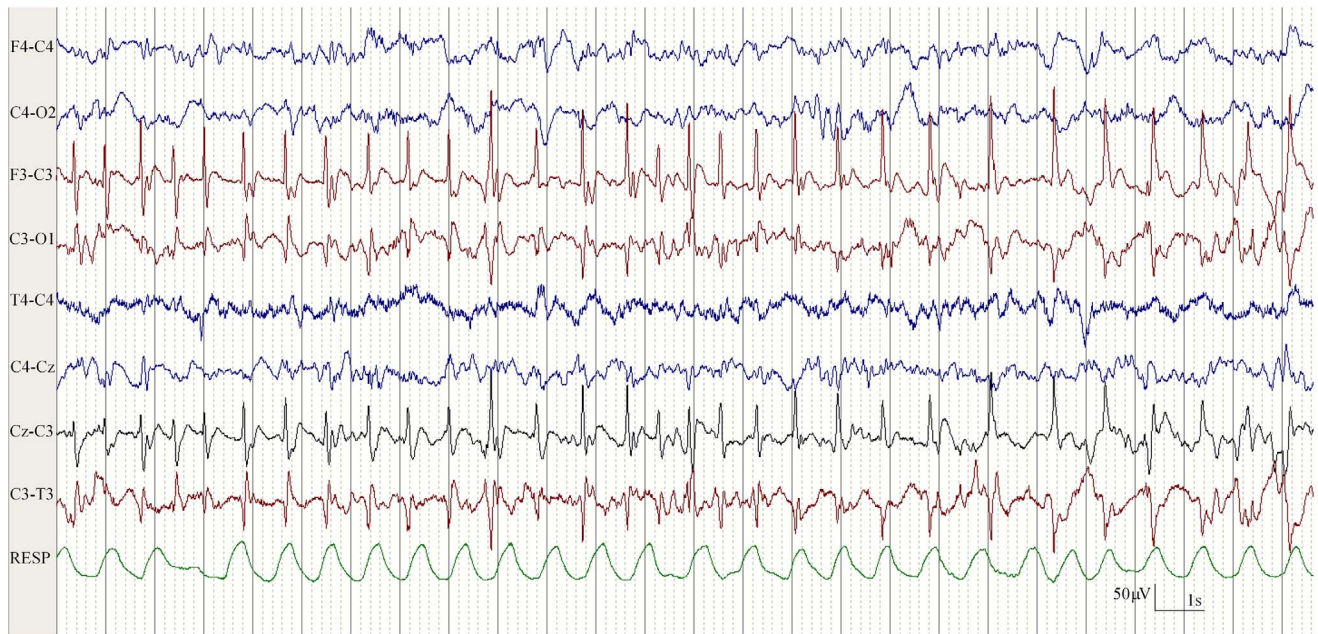
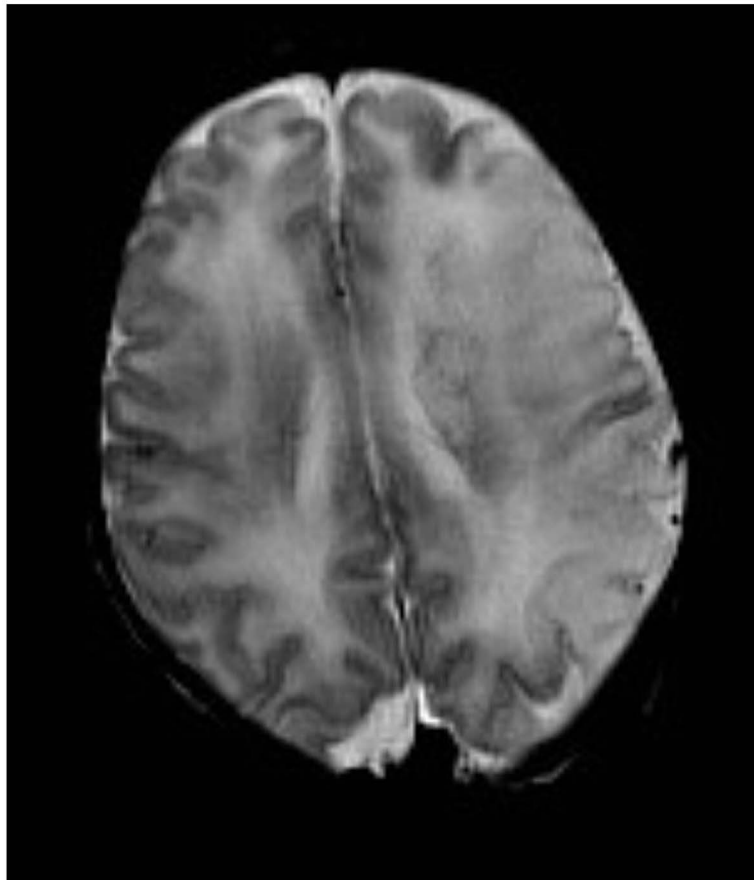
A**B**

Figure 2. A. EEG in a neonate (case 6). Seizures arising from the left hemisphere corresponding with a left middle cerebral artery infarction on cranial MRI. **B. Cranial MRI in a neonate (case 6).** The sequence is an axial T2 turbo spin echo performed on day 7 of life. Note the characteristic focal spike and wave discharges over the left hemisphere with phase reversal over the left central region.
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Table 2. Characteristics of EEG and seizures.

| Neonate | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|--|--|---|--|--|--|--|---|--|
| Summary of background EEG feature | | | | | | | | | |
| Continuous activity | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Symmetry | Left mild suppression | Left significant suppression | Right mild suppression | Good | Right mild suppression | Good | Good | Left significant suppression | Right mild suppression |
| Intermittent features | Left-sided sharp theta bursts | Left-sided theta sharp waves | Right focal sharp waves | Left-sided sharp waves in quiet sleep | Right-sided theta sharp waves | Left-sided theta sharp waves | Left-sided focal sharp theta waves | Left-sided theta sharp waves | Right-sided sharp waves |
| Sleep cycling | Normal bilaterally | Disturbed unilaterally | Disturbed bilaterally | Disturbed bilaterally | Normal bilaterally | Normal bilaterally | Disturbed unilaterally | Disturbed unilaterally | Normal bilaterally |
| Seizure morphology | Focal spikes over left central with phase reversal | Focal spikes over left central with phase reversal | Focal spikes over right central with phase reversal | Focal spikes over left central with phase reversal | Focal spikes & polyspikes over right central with phase reversal | Focal spikes over left central with phase reversal | Focal spikes over left central with phase reversal | Focal spikes & polyspikes over left central with phase reversal | Focal spikes & polyspikes over right central with phase reversal |
| Summary of seizure burden | | | | | | | | | |
| Total seizure burden (minutes) | 19 | 67 | 101 | 133 | 162 | 201 | 266 | 327 | 332 |
| Seizure burden (minutes/hour) | 2.70 | 7.28 | 27.60 | 5.53 | 10.27 | 18.15 | 12.77 | 9.25 | 6.18 |
| Mean seizure duration (seconds) | 370 | 98 | 356 | 362 | 120 | 523 | 143 | 195 | 146 |
| Seizure window (hours) | 7 | 9 | 4 | 24 | 16 | 11 | 21 | 35 | 54 |
| Status epilepticus | None | None | Yes | None | None | Yes | Yes | Yes | Yes |
| Number of seizures (n) | 3 | 41 | 17 | 22 | 81 | 23 | 112 | 101 | 136 |
| Seizure classification | | | | | | | | | |
| Electrographic-only seizures: n (%) | 0 (0) | 27 (66) | 8 (47) | 20 (91) | 77 (95) | 13 (57) | 77 (69) | 62 (61) | 121 (89) |
| Electrographic-only seizure burden: minutes (%) | 0 (0) | 28 (42) | 26 (25) | 129 (97) | 146 (90) | 74 (37) | 129 (49) | 244 (74) | 282 (85) |
| Electroclinal seizures: n (%) | 2 (66) | 10 (24) | 7 (41) | 1 (4.5) | 3 (3.7) | 9 (39) | 32 (29) | 35 (35) | 15 (11) |
| Electroclinal seizure burden: minutes (%) | 18 (95) | 30 (44) | 48 (48) | 3 (2) | 15 (9) | 108 (54) | 126 (47) | 80 (24) | 50 (15) |
| Clonic/subtle seizures: n | 2/0 | 0/10D | 5/2C | 1/0 | 3/0 | 0/9S | 17/15S | 16/19Y | 9/6M |
| Video obscured: n (%) | 1 (33) | 4 (10) | 2 (12) | 1 (4.5) | 1 (1.3) | 1 (4) | 3 (2) | 4 (4) | 0 (0) |

Subtle seizures: C, cycling movements of the limbs; D, desaturations; M, mouthing and smacking; S, sucking; Y, yawning.

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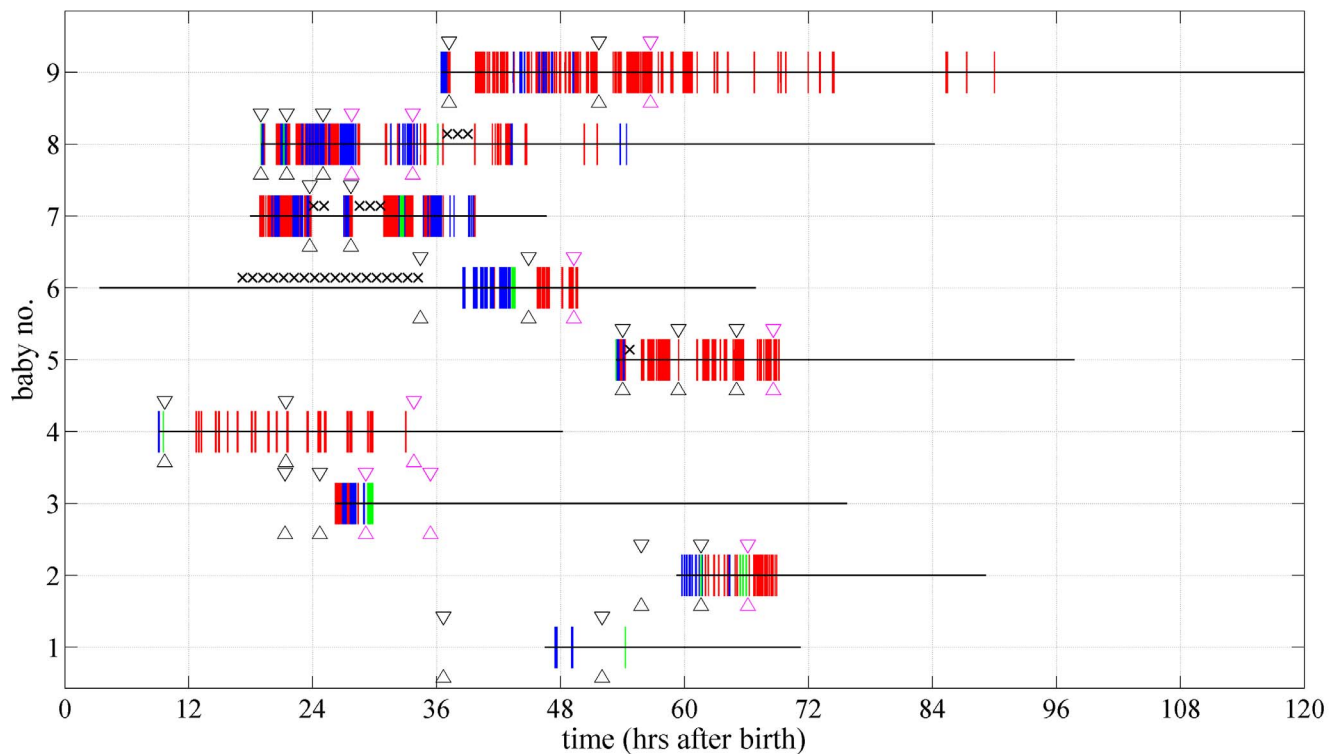


Figure 3. Characteristics of seizures and anticonvulsant administration in each neonate. Vertical red lines denote the presence of electrographic-only seizures, vertical blue lines denote electroclinical seizures and vertical green lines denote obscured seizures. Horizontal black line denotes the period of EEG monitoring. Black crosses denote missing data. Timepoints bounded by black arrows denote the first-line anticonvulsant administration while the magenta arrows denote the second-line anticonvulsant administration.
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parietal areas). [25] Clinical signs may not manifest if the motor cortical strip is not involved. [25] All neonates in our study had some degree of MCA involvement; at some timepoint a clinical correlate (often very subtle) was evident. Although typically neonates with PAIS are non-encephalopathic, [26] hypotonia, poor sucking reflex and irritability have been described. [27] Subtle seizures in our cohort involved mainly oral-buccal-lingual movements (four of six neonates); this is in line with other studies. [2], [28] In PAIS, autonomic dysfunction such as apnoeic spells [29], [30] has been reported in up to 36% of neonates; [31] only one neonate in our study presented with apnoea before any anticonvulsant administration. Other subtle seizures which have been previously described included eye blinking, vertical nystagmus and thumb adduction, [29] but multichannel EEG monitoring was not applied, thus the accuracy of these clinical signs is unknown. Our results support the suggestion for low threshold in initiating EEG monitoring when there is any suspicion of unusual movements which may be seizures.

To date, reported incidences of seizures in neonates with PAIS are mainly based on observation of neonatal behaviours, [22], [32] rather than on multichannel EEG which is the gold standard for accurate detection of neonatal seizures. [18], [33–35] Approximately 20% of neonatal seizures in term neonates are due to PAIS. [2] Conversely, while neonatal seizures have been noted in 26% of neonates with PAIS, [3] we believe these numbers could be much higher if detection of seizures is based on prolonged multichannel EEG monitoring. A limitation of our study is the small number of neonates with PAIS. In our cohort of neonates, all except one neonate (case 2) was captured when they presented with

hemiconvulsions before discharge shortly after birth in our 2 neonatal units. We only included neonates that presented with clear PAIS involving at most 2 arterial territories and who had continuous multichannel EEG monitoring as soon as possible after their presentation with seizures. While being monitored, these neonates with seizures showed asymmetrical characteristics on the EEG. In this period, other neonates would have presented but did not have continuous EEG monitoring undertaken. It is difficult to diagnose all neonates with PAIS in the neonatal period as the majority of term neonates affected by PAIS are asymptomatic; [1] appearing clinically well enough to be sent to the postnatal ward shortly after birth. In our 2 units, there is a policy of early maternal and neonatal discharge. Any neonate presenting with seizures after they were discharged would have been readmitted to regional paediatric hospitals, not the neonatal units. Even though our number of neonates with PAIS is small, we believe that the novelty here is having captured a number of neonates who had early and long duration of multichannel EEG monitoring.

In our study, EEG monitoring was initiated only after clinical seizures were observed in the first 3 days of life; we have shown that the age of first clinical seizure and first recorded EEG seizure [33 (17–42) and 36 (19–54) hours] were within 72 hours of age. This is current practice in most neonatal units as there are no existing early indicators to identify neonates with PAIS, hence it is possible that neonates with PAIS and electrographic-only seizures may have been missed during our recording period. Early EEG monitoring may have a role in providing an early indicator of PAIS, as early EEG from three hours after delivery has been shown to demonstrate occasional focal sharp waves over the

infarcted region which became more frequent, complex and of higher amplitude in quiet sleep. [36]

In conclusion, EEG in neonates with PAIS demonstrated distinctive features in the background EEG and morphology of seizures. These features were present from very early after birth. Given the ease with which EEG monitoring can now be performed at the bedside, careful EEG analysis may prove very useful for early diagnosis of PAIS. For the first time, we have also quantified the seizure burden in neonates with PAIS using multichannel video-EEG. The majority of seizures in neonates with PAIS will escape detection without prolonged multichannel EEG monitoring.

References

- Lynch JK, Nelson KB (2001) Epidemiology of perinatal stroke. *Curr Opin Pediatr* 13: 499–505.
- Volpe JJ (2008) Neonatal Seizures. In: *Neurology of the Newborn*. Philadelphia, PA: WB Saunders Company. pp. 203–244.
- Rafay MF, Cortez MA, de Veber GA, Tan-Dy C, Al-Futaisi A, et al. (2009) Predictive value of clinical and EEG features in the diagnosis of stroke and hypoxic ischemic encephalopathy in neonates with seizures. *Stroke* 40: 2402–2407.
- Ramaswamy V, Miller SP, Barkovich AJ, Partridge JC, Ferriero DM (2004) Perinatal stroke in term infants with neonatal encephalopathy. *Neurology* 62: 2088–2091.
- Rutherford MA, Ramenghi LA, Cowan FM (2012) Neonatal stroke. *Arch Dis Child Fetal Neonatal Ed* 97:F377–F384.
- Cowan F, Mercuri E, Groenendaal F, Bassi L, Ricci D, et al. (2005) Does cranial ultrasound imaging identify arterial cerebral infarction in term neonates? *Arch Dis Child Fetal Neonatal Ed* 90:F252–F256.
- van Rooij LG, de Vries LS, van Huffelen AC, Toet MC (2010) Additional value of two-channel amplitude integrated EEG recording in full-term infants with unilateral brain injury. *Arch Dis Child Fetal Neonatal Ed* 95:F160–F168.
- Mercuri E, Rutherford M, Cowan F, Pennock J, Counsell S, et al. (1999) Early prognostic indicators of outcome in infants with neonatal cerebral infarction: a clinical, electroencephalogram, and magnetic resonance imaging study. *Pediatrics* 103: 39–46.
- Klem GH, Luders HO, Jasper HH, Elger C (1999) The ten-twenty electrode system of the International Federation. *The International Federation of Clinical Neurophysiology. Electroencephalogr Clin Neurophysiol Suppl* 52: 3–6.
- Lamblin MD, Walls EE, Andre M (2013). The electroencephalogram of the full-term newborn: review of normal features and hypoxic-ischemic encephalopathy patterns. *Neurophysiol Clin* 43: 267–287.
- Clancy RR (2006) Prolonged electroencephalogram monitoring for seizures and their treatment. *Clin Perinatol* 33: 649–665.
- Ortibus EL, Sum JM, Hahn JS (1996) Predictive value of EEG for outcome and epilepsy following neonatal seizures. *Electroencephalogr Clin Neurophysiol* 98: 175–185.
- Weiner SP, Painter MJ, Geva D, Guthrie RD, Scher MS (1991) Neonatal seizures: electroclinical dissociation. *Pediatr Neurol* 7: 363–368.
- Marks MP, Holmgren EB, Fox AJ, Patel S, von Kummer R, et al. (1999) Evaluation of early computed tomographic findings in acute ischemic stroke. *Stroke* 30: 389–392.
- Lynch N, Low E, Rennie JM, Boylan GB (2011). Comparison of seizure characteristics in full term neonates with stroke and hypoxic-ischemic encephalopathy. *Arch Dis Child* 96:A39.
- Mercuri E (2001) Early diagnostic and prognostic indicators in full term infants with neonatal cerebral infarction: an integrated clinical, neuroradiological and EEG approach. *Minerva Pediatr* 53: 305–311.
- Clancy RR, Legido A (1987) The exact ictal and interictal duration of electroencephalographic neonatal seizures. *Epilepsia* 28: 537–541.
- Murray DM, Boylan GB, Ali I, Ryan CA, Murphy BP, et al. (2008) Defining the gap between electrographic seizure burden, clinical expression and staff recognition of neonatal seizures. *Arch Dis Child Fetal Neonatal Ed* 93:F187–F191.
- Glykys J, Dzhalal VI, Kuchibhotla KV, Feng G, Kuner T, et al. (2009) Differences in cortical versus subcortical GABAergic signaling: a candidate mechanism of electroclinical uncoupling of neonatal seizures. *Neuron* 63: 657–672.
- Vasiljevic B, Magljalic-Djukic S, Gojnic M (2012) The prognostic value of amplitude-integrated electroencephalography in neonates with hypoxic-ischemic encephalopathy. *Vojnosanit Pregl* 69: 492–499.
- Estan J, Hope P (1997) Unilateral neonatal cerebral infarction in full term infants. *Arch Dis Child Fetal Neonatal Ed* 76:F88–F93.
- Golomb MR, Garg BP, Carvalho KS, Johnson CS, Williams LS (2007) Perinatal stroke and the risk of developing childhood epilepsy. *J Pediatr* 151: 409–413.
- Rando T, Ricci D, Mercuri E, Frisone MF, Luciano R, et al. (2000) Periodic lateralized epileptiform discharges (PLEDs) as early indicator of stroke in full-term newborns. *Neuropediatrics* 31: 202–205.
- Boylan GB, Rennie JM, Pressler RM, Wilson G, Morton M, et al. (2002) Phenobarbitone, neonatal seizures, and video-EEG. *Arch Dis Child Fetal Neonatal Ed* 86:F165–F170.
- Govaert P, Matthys E, Zecic A, Roelens F, Oostra A, et al. (2000) Perinatal cortical infarction within middle cerebral artery trunks. *Arch Dis Child Fetal Neonatal Ed* 82:F59–F63.
- Cowan F, Rutherford M, Groenendaal F, Eken P, Mercuri E, et al. (2003) Location and timing of brain lesions in term infants with neonatal encephalopathy. *Lancet* 361: 736–742.
- Miller V (2000) Neonatal cerebral infarction. *Semin Pediatr Neurol* 7: 278–288.
- Pinto LC, Giliberti P (2001) Neonatal seizures: background EEG activity and the electroclinical correlation in full-term neonates with hypoxic-ischemic encephalopathy. Analysis by computer-synchronized long-term polygraphic video-EEG monitoring. *Epileptic Disord* 3: 125–132.
- Fujimoto S, Yokochi K, Togari H, Nishimura Y, Inukai K, et al. (1992) Neonatal cerebral infarction: symptoms, CT findings and prognosis. *Brain Dev* 14: 48–52.
- Hoogstraate SR, Lequin MH, Huysman MA, Ahmed S, Govaert PP (2009) Apnoea in relation to neonatal temporal lobe haemorrhage. *Eur J Paediatr Neurol* 13: 356–361.
- Sreenan C, Bhargava R, Robertson CM (2000) Cerebral infarction in the term newborn: clinical presentation and long-term outcome. *J Pediatr* 137: 351–355.
- Kirton A, Armstrong-Wells J, Chang T, Devere G, Rivkin MJ, et al. (2011) Symptomatic neonatal arterial ischemic stroke: the International Pediatric Stroke Study. *Pediatrics* 128:e1402–e1410.
- Glass HC, Wirrell E (2009) Controversies in neonatal seizure management. *J Child Neurol* 24: 591–599.
- Low E, Boylan GB, Mathieson SR, Murray DM, Korotchkova I, et al. (2012) Cooling and seizure burden in term neonates: an observational study. *Arch Dis Child Fetal Neonatal Ed* 97:F267–F272.
- Wusthoff CJ, Dlugos DJ, Gutierrez-Colina A, Wang A, Cook N, et al. (2011) Electrographic seizures during therapeutic hypothermia for neonatal hypoxic-ischemic encephalopathy. *J Child Neurol* 26: 724–728.
- Walsh BH, Low E, Bogue CO, Murray DM, Boylan GB (2011) Early continuous video electroencephalography in neonatal stroke. *Dev Med Child Neurol* 53: 89–92.

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Author Contributions

Conceived and designed the experiments: GBB JMR. Performed the experiments: EL GBB SRM. Analyzed the data: EL GBB SRM NJS. Contributed reagents/materials/analysis tools: EL GBB SRM NJS VL. Wrote the paper: EL SRM NJS VL CAR COB JMR GBB. Provided reports and measurements on the cranial imaging scans: COB.

Short-term Effects of Phenobarbitone on Electrographic Seizures in Neonates

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33 Abstract

34 **Background:** Phenobarbitone is the most common first-line anti-seizure drug and is effective in approximately 50% of all
35 neonatal seizures. **Objective:** To describe the response of electrographic seizures to administration of intravenous
36 phenobarbitone in neonates using seizure burden analysis techniques. **Methods:** Multi-channel conventional EEG, reviewed by
37 experts, was used to determine the electrographic seizure burden in hourly epochs. The maximum seizure burden (MSB)
38 evaluated one hour before each phenobarbitone dose (T-1) was compared to seizure burden in periods of increasing duration
39 after each phenobarbitone dose was administered (T+1, T+2 to seizure offset). Differences were analyzed using linear mixed
40 models and summarized as means and 95% confidence intervals. **Results:** Nineteen neonates had electrographic seizures and
41 met the inclusion criteria for the study. The MSB was significantly reduced one hour after the administration of phenobarbitone
42 (T+1) [-14.0 (95% confidence interval: -19.6, -8.5) minutes per hour; $p<0.001$]. The percentage reduction was 74 (36-100)%. This
43 reduction was temporary and not significant within four hours of administering phenobarbitone. Subgroup analysis showed
44 that only phenobarbitone doses at 20 mg/kg resulted in a significant reduction in the MSB from T-1 to T+1 ($p=0.002$).
45 **Conclusions:** Phenobarbitone significantly reduced seizures within one hour of administration as assessed with continuous
46 multi-channel EEG monitoring in neonates. The reduction was not permanent and seizures were likely to return within four
47 hours of treatment in most neonates.

49 Introduction

50 Seizures are harmful to the developing neonatal brain [1], are a neurological emergency and require prompt treatment with an
51 anti-seizure drug (ASD). In 2011, published management guidelines for neonatal seizure by the World Health Organisation
52 strongly recommended only the use of phenobarbitone as a first-line ASD; however it was acknowledged that this
53 recommendation was based on very low quality evidence [2]. To date, phenobarbitone remains the most common first-line
54 ASD for treatment of neonatal seizures; this practice is largely based on tradition, local protocols or personal preference as
55 phenobarbitone has been shown to abolish seizures in only 50% of cases [3,4]. As a result, the treatment of neonatal seizures
56 has not changed significantly in the last 50 years, although a number of potential new treatments are being investigated [5].

58 The development of ASDs for neonates remains a challenging area due to developmental differences between the neonatal and
59 adult brain such as higher concentrations of intracellular chloride and a lower expression of gamma-aminobutyric acid (GABA)
60 receptors in the developing neonatal brain [6]. Evidence on the effectiveness of ASDs for neonates is translated from studies in
61 older children and animal models. There is also inconsistency on the measurement of effectiveness of ASDs. Effectiveness
62 assessed by clinical observation is known to be inaccurate [7,8], whilst others have used the amplitude-integrated EEG (aEEG)
63 [9] which has limitations for seizure detection in neonates [10]. Effectiveness is often defined as a binary variable (effective vs.
64 ineffective) without more detailed quantification of the actual reduction of electrographic seizures [9,11] or the duration of the
65 effect. The complete response of electrographic seizures to individual doses of ASDs, therefore, remains poorly understood in
66 neonates. We aimed to measure the effectiveness of individual phenobarbitone doses for the reduction of seizures using multi-
67 channel EEG recordings and detailed seizure burden analysis in a cohort of term neonates with mixed seizure aetiology.

69 Methods

70 As part of an ongoing study of neonatal seizures, neonates were enrolled from the neonatal intensive care units in Cork
71 University Maternity Hospital, Ireland and University College London Hospital, United Kingdom from January 2009 to October
72 2011. Neonates ≥ 37 weeks gestation were enrolled for EEG monitoring if there was any evidence of encephalopathy or seizures
73 within 72 hours of age. Neonates who had at least one ASD dose administered during electrographic seizures were included in
74 the study. Neonates were excluded if *all* their phenobarbitone doses were administered without electrographic evidence of
75 seizures. Institutional review board approval was obtained from the Clinical Research Ethics Committees of the Cork Teaching
76 hospitals, Ireland and the National Health Service in the United Kingdom, via the Integrated Research Application Service.
77 Written, informed consent was obtained from at least one parent of each neonate who participated in this study.

All clinical seizures and EEG seizures recognized by the clinical team were treated. The standardized protocol for ASD usage was similar in both hospitals. At the discretion of the attending neonatologist, a phenobarbitone loading dose of 10 or 20 mg/kg was administered intravenously on seizure recognition. Subsequent phenobarbitone doses up to an accumulated dosage of 40 mg/kg or a second-line (intravenous phenytoin or midazolam), third and fourth-line ASDs were administered if clinical and/or electrographic seizures recurred. The time, dose and accumulated dosage of phenobarbitone were recorded. We assumed a zero clearance rate when calculating the accumulated dosage as the majority of doses were given well within the half-life of phenobarbitone [9,11,12]. At both hospitals, EEG recording methods were identical. A Nicolet monitor (CareFusion NeuroCare, Wisconsin, USA) was used to record multi-channel video-EEG using the 10-20 system of electrode placement modified for neonates [13]. The entire EEG recording from each neonate was independently reviewed by two experienced neonatal electroencephalographers (GBB, SRM) who were blinded to clinical details. Each electrographic seizure annotated was defined according to Clancy *et al.* [14] and status epilepticus was defined as by Ortibus *et al.* [15].

We calculated the hourly seizure burden (HSB) in each one hour period of the EEG recording based on the electrographic seizure annotations. This was defined as the accumulated seizure duration within a one hour window, shifted across the EEG monitoring period with a one minute interval (fig. 1). In each neonate, the maximum HSB (MSB) was used to assess the effectiveness of phenobarbitone. The MSB in a time period one hour before a phenobarbitone dose (T_{-1}) was compared to a time period of one hour in duration beginning immediately after cessation of the phenobarbitone infusion which was completed in 30 minutes. The MSB in time periods of increasing duration was also used to assess the duration of the effect of phenobarbitone administration. Time periods were increased from one hour (T_{+1}), in hourly increments (T_{+2} , T_{+3} , T_{+4}) until the last electrographically recorded seizure (T_{+LR}). For example, T_{-1} is a time period from 1 hour before the start of phenobarbitone infusion until the start of phenobarbitone infusion and T_{+3} is a time period from after cessation of phenobarbitone infusion until 3 hours after the cessation of phenobarbitone infusion (fig. 1). Furthermore, the seizure burden between seizure onset and phenobarbitone administration was compared between doses which showed a complete (MSB=0) or incomplete (MSB>0) effect.

Statistics

Continuous variables were described using median [interquartile ranges (IQR)] and categorical variables using frequencies. Differences between MSB before and after phenobarbitone administration were calculated for each period (T_{+1} , T_{+2} until T_{+LR}). Linear mixed models with a neonate-level random effect were used to account for possible correlations among observations from the same neonate (more than one phenobarbitone dose per neonate). For comparisons between groups, group was included as a fixed effect in the linear mixed model. Results based on linear mixed models were presented as means [95% confidence intervals (CI)]. The comparison between MSB pre- and post-one hour (T_{-1} vs. T_{+1}) was also performed in subgroups defined by dosage and accumulated dosage. We denote the number of neonates as n_n and the number of doses as n_d . All statistical analyses were performed in SAS 9.3 (SAS Institute Inc., Cary, NC, USA) and $p<0.05$ were considered as statistically significant.

Results

During the study period, of the 35 neonates with electrographic seizures identified, sixteen did not meet the inclusion criteria for ASD analysis (two neonates received no ASD and 14 were treated before EEG monitoring commenced or when there were no accompanying electrographic seizures). Therefore, the effectiveness of phenobarbitone was measured in the remaining 19 neonates; table 1 lists their clinical characteristics and details of seizure burden. EEG monitoring began at median (IQR) age of 17 (4-36) hours, EEG duration was 78 (56-109) hours and the age of first electrographic seizure was 18 (11-41) hours.

The 19 neonates received a total of 37 loading phenobarbitone doses during EEG monitoring, 31 of which were given during electrographic seizures. The median (IQR) time between seizure onset and phenobarbitone administration was 3.3 (1.1-10.7 hour); $n_d=31$. A significant MSB reduction was seen in the hour immediately after phenobarbitone administration [mean

125 difference (95% CI): -14.0 (-19.6, -8.5) minutes/hour; $p < 0.001$ ($n_n=19$; $n_d=31$) (table 2). The median (IQR) percentage
 126 reduction was 74.0 (36.0-100.0)% in the 31 doses. In 13 of the 19 neonates, a complete abolition of electrographic seizures was
 127 seen in the first hour following a loading phenobarbitone dose at 20 mg/kg. This abolition was permanent in 3 neonates. The
 128 MSB did not show a significant reduction over the longer term when comparing MSB in T_{-1} to T_{+LR} [mean difference (95% CI): -
 129 2.3 (-9.2, 4.5) minutes/hour; $p=0.481$]. In fact, the MSB was not significantly reduced after phenobarbitone by T_{+4} (table 3). The
 130 seizure burden before phenobarbitone administration was significantly lower for doses which resulted in a complete MSB
 131 reduction in the first hour (T_{+1}) [mean (95% CI): 28.1 (-5.9, 62.1) minutes ($n_d=13$)] compared to doses which did not completely
 132 reduce the MSB [mean (95% CI): 117.6 (71.3, 164.0) minutes ($n_d=7$); $p=0.004$]. Ten of 13 doses which resulted in complete
 133 seizure reduction in T_{+1} were a first dose (table 2). The median (IQR) time between electrographic seizure onset and first
 134 analyzed dose was 1.8 (0.7-2.4) hours.

135
 136 The MSB reduction was greater when 20 mg/kg was administered compared to a dose of 10 mg/kg [mean difference (95% CI)
 137 of 20 mg/kg ($n_d=20$) vs. 10 mg/kg ($n_d=11$): -18.6 (-23.7, -13.5) vs. -4.4 (-11.3, 2.5) minutes/hour; $p=0.002$]. In fact, 20 of 20
 138 (100%) doses of phenobarbitone at 20 mg/kg resulted in a reduction in MSB during T_{+1} and in 13 of 20 (65%) doses, the MSB
 139 was zero during T_{+1} . This result is reflected when observing the effect of accumulated dosage as the MSB reduction in T_{+1} was
 140 significantly higher for accumulated doses of 20 mg/kg [mean difference (95% CI): -19.0 (-25.0, -12.9) minutes/hour ($n_d=14$)]
 141 and 40 mg/kg [mean difference (95% CI): -14.3 (-21.3, -7.4) minutes/hour ($n_d=10$)] compared to an accumulated dose of 30
 142 mg/kg [mean difference (95% CI): -1.0 (-10.0, 8.0) minutes/hour ($n_d=6$); $p=0.001$ and 0.017 respectively (fig. 2).

143
 144 A total of 13 doses of additional ASDs were given to neonates who received phenobarbitone as a first-line ASD (phenytoin,
 145 midazolam, clonazepam). Only one dose of phenobarbitone at 20 mg/kg was administered after the administration of second-
 146 line ASD (<1 hour after phenytoin). No second or third-line ASDs were given in T_{+1} . The median time between phenobarbitone
 147 administration and additional lines of ASD administration was 8.3 (5.2-12.4) hour.

148 149 Discussion

150 We have shown that a loading dose of phenobarbitone results in an immediate but temporary reduction in electrographic
 151 seizure burden in most term neonates; seizures returned to pre-treatment levels within four hours of administration. We have
 152 also shown that 20 mg/kg doses were more effective than 10 mg/kg, and that phenobarbitone was more likely to abolish
 153 seizures in the short-term if given before a large accumulation of seizures was apparent.

154
 155 Phenobarbitone, a barbiturate, primarily has an inhibitory effect in the adult brain by prolonging the action of GABA, acting
 156 mainly on the GABA_A receptors [6]. The purported effect of phenobarbitone (seizure cessation via GABA agonism) is somewhat
 157 problematic given the large body of evidence suggesting that GABA is excitatory in the neonatal brain [16,17]. This excitatory
 158 drive may be due to the predominance of the sodium-potassium-chloride cotransporter isoform 1 which moves chloride into
 159 the cell and the lower expression of potassium-chloride cotransporter isoform 2 which moves chloride out of the cell [6]. This
 160 suggests, and has been shown in animal models, that a GABA agonist will facilitate seizures in the developing neonatal brain.
 161 However, GABA antagonists do not reduce seizures [18] and phenobarbitone abolishes seizures in 50% of cases [3,4]. We have
 162 shown that phenobarbitone abolishes seizures during T_{+1} in 65% of cases when the dose is 20 mg/kg. Conflicting results
 163 between animal and clinical studies must be resolved in order to develop improved treatment strategies for neonatal seizures.

164
 165 We have shown that the effectiveness of phenobarbitone is temporary and the reduction in seizure burden is limited beyond
 166 four hours of administration. This is conspicuously shorter than the pharmacokinetic half-life of phenobarbitone (range: 45 to
 167 500 hours [12] and is variable depending on circumstances [9,11]). Phenobarbitone resistance can occur in the neonatal brain
 168 and a change in GABA_A receptor subunit, activation of a non-functional 'spare' GABA_A receptor, uncoupling of receptors and
 169 post-translational modification of GABA_A receptor have been hypothesized as possible mechanisms for this
 170 pharmacoresistance [19]. We have also shown that doses of phenobarbitone were more likely to abolish seizures in the short-

term if they were given before a large accumulation of seizures has occurred. This implies that rapid identification of seizures and intervention, when the accumulated seizure duration is low, may be more beneficial. Nardou *et al.* hypothesized that the increase in intracellular chloride levels during recurrent seizures may enhance the excitatory component of GABA, causing GABA agonists such as phenobarbitone to be ineffective in reducing seizure burden [20].

Seizures are intermittent and highly variable and show a natural tendency to decay after a long period of time [21]. Up to 80% of neonatal seizures may be missed using clinical observation alone; methods such as the aEEG are unreliable and dissociation of electroclinical seizures increases after ASD usage [7,8,10,14,22]. It is not surprising that many seizures were treated before monitoring began or when no electrographic seizures were evident.

In maternity units like ours, which monitor neonates with seizures intensively, we still found suboptimal treatment of neonatal seizures. If electrographic seizures emerged during out-of-hours, there were no alarm systems to alert clinical teams to ongoing electrographic seizures [23], hence treatment was not always instigated promptly. We are aware that this is a heterogeneous group and while the numbers (n=19) in our study were sufficient for the general assessment of phenobarbitone effectiveness on neonatal seizures, it was insufficient for assessment of phenobarbitone effectiveness with respect to seizure aetiology, dosing strategies or therapeutic hypothermia. The presence of second and third-line ASDs would have resulted in a possible underestimate of the MSB in longer duration post-phenobarbitone time periods. This, however, would not change our conclusion on the short-term effect of phenobarbitone as only 1 dose of phenobarbitone was given in the presence of a second-line ASD. In fact, an underestimation of MSB only enhances our findings relating to a short-term reduction in seizures and that seizure re-occurrence is earlier than can be accounted for by biological clearance.

Conclusion

Phenobarbitone immediately reduced the accumulation of seizures in a cohort of neonates with mixed aetiology. The effect was temporary and the reduction in seizure burden was not significant within four hours of treatment. Doses of phenobarbitone at 20 mg/kg as subsequent dose after the initial loading dose of 20 mg/kg, rather than 10 mg/kg as subsequent doses were significantly more effective in reducing seizure burden. Phenobarbitone was also more effective if administered when the seizure burden was relatively low.

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Conflict of Interest: None of the authors has any conflict of interest to disclose.

Ethics: The parents of babies have given their informed consent and that the study protocol has been approved by the institute's committee on human research. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Table 1. Summary characteristics of the 19 neonates included for the study analysis

| | |
|---|---------------------------|
| Age at EEG monitoring (hours) | 17 (4-36) |
| Age of first EEG seizure (hours) | 18 (11-41) |
| Duration of EEG monitoring (hours) | 78 (56-109) |
| Summary of seizure burden | |
| Recorded seizure burden (minutes) | 119 (45-305) |
| Seizure number (n) | 25 (11-130) |
| Mean seizure duration (seconds) | 183 (126-298) |
| Neonates with status epilepticus (n) | 9 |
| Neonates who received therapeutic hypothermia (n) | 7 |
| Age of first anti-seizure drug (hours) | 19 (11-51) |
| Clinical diagnosis | Number of neonates |
| HIE grade II | 5 (3 were cooled) |
| HIE grade III | 5 (4 were cooled) |
| Multiple infarction | 2 |
| Focal arterial infarction | 2 |
| Bifocal arterial infarction | 2 |
| Suspected viral encephalitis | 1 |
| Unknown cause | 1 |
| Benign non-familial seizures | 1 |

Data are expressed as n or median (interquartile).

HIE: hypoxic-ischaemic encephalopathy.

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| Table 3. Results of linear mixed models for maximum hourly seizure burden (MSB) post and pre-one hour of phenobarbitone administration from 31 observations at each timepoint across the 19 neonates | | |
|---|--|--------------|
| MSB: Post-phenobarbitone administration | Mean difference (95% confidence interval) in minutes/ hour | p-values |
| Post: 1 hour | -14.04 (-19.60 to -8.48) | <0.001 |
| Post: 2 hours | -9.48 (-15.05 to -3.91) | 0.003 |
| Post: 3 hours | -7.53 (-13.34 to -1.73) | 0.016 |
| Post: 4 hours | -5.38 (-11.15 to 0.39) | 0.064 |
| Post: 5 hours | -4.89 (-10.70 to 0.93) | 0.089 |
| Post: 6 hours | -4.99 (-10.88 to 0.91) | 0.090 |
| Post: 7 hours | -3.39 (-9.78 to 2.99) | 0.268 |
| Post: 8 hours | -3.29 (-9.78 to 3.21) | 0.292 |
| Post: 9, 10, 11 hours | -2.92 (-9.31 to 3.46) | 0.338 |
| Post: 12 hours | -2.92 (-9.31 to 3.47) | 0.338 |
| Until T_{+LR} | -2.33 (-9.20 to 4.54) | 0.481 |

Figure 1.

An example plot of the hourly seizure burden (HSB) over time (blue line) for one neonate with seizures overlaid with the time periods used to assess the effectiveness of phenobarbitone. The change in HSB was compared one hour before (T_{-1}), one hour after (T_{+1}) and in the remaining hours of electrographic recorded seizures after the administration of phenobarbitone (T_{+LR}). The upper plot is the complete seizure time course for the neonate with T_{+LR} (black horizontal lines) shown for each 20 mg/kg dose of phenobarbitone (red vertical lines). After the second dose of phenobarbitone was given, electroclinical dissociation of seizures occurred and subsequent seizures were not highlighted to the clinical team so there was no additional ASD given for seizures between 30-40 hours. The lower plot is the magnified version of the upper plot with T_{-1} (red boxes) and T_{+1} (black boxes) shown for each dose of phenobarbitone (red vertical lines). There is a clear reduction in maximum HSB between T_{-1} and T_{+1} following the administration of each dose of phenobarbitone and these seizures return within T_{+LR} . Note that some smoothing is apparent in the HSB as both future and past values are used to estimate the HSB and a 30 minute delay is taken into account for phenobarbitone infusion.

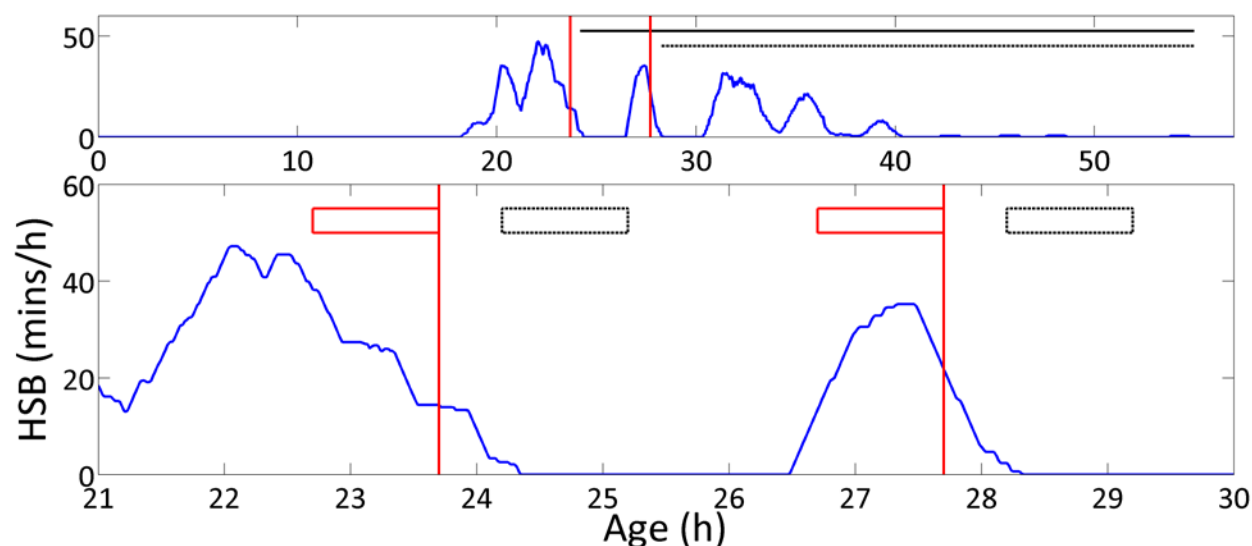
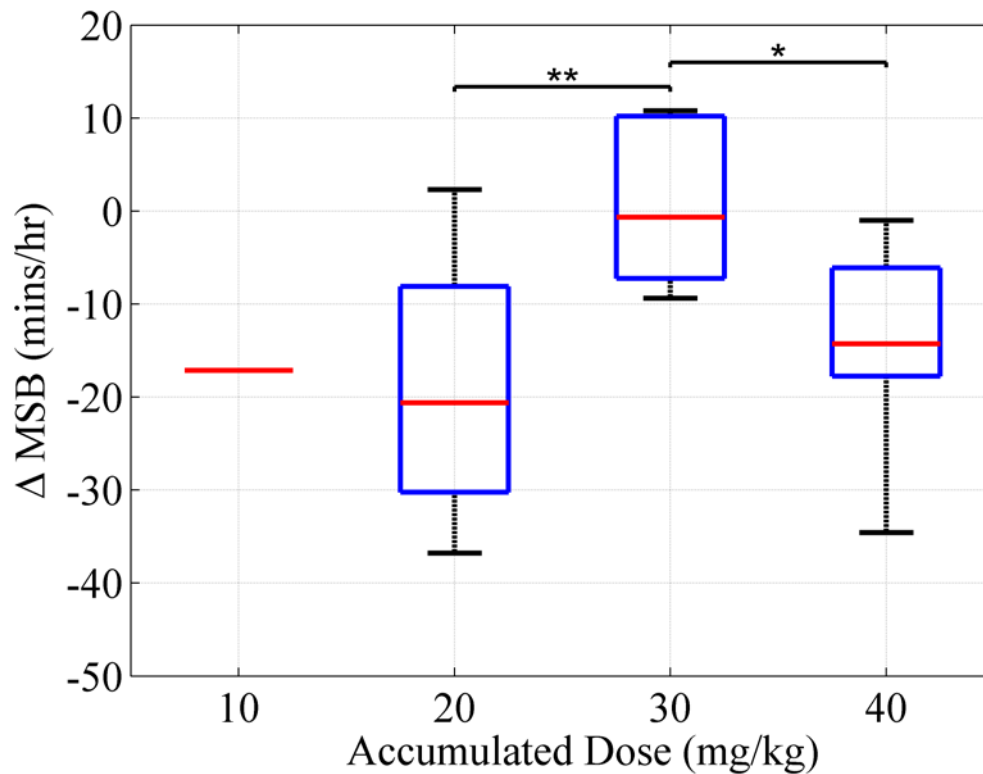


Figure 2.

The short term reduction in seizure burden of phenobarbitone associated with accumulated dosage. Δ MSB denotes the change in MSB between time periods T_{+1} and T_{-1} . A negative Δ MSB implies a reduction in seizure burden between T_{-1} and T_{+1} . The Δ MSB at accumulated doses of 20 mg/kg and 40 mg/kg are significantly lower than accumulated doses of 30 mg/kg. Only 1 neonate had a dose of 10 mg/kg as a first dose (accumulated dosage of 10 mg/kg).



References

- [1] Thibeault-Eybalin MP, Lortie A, Carmant L: Neonatal seizures: do they damage the brain? *Pediatr Neurol* 2009;40:175-180.
- [2] WHO. Guidelines on neonatal seizures. Geneva: World Health Organization. 2011.
- [3] Boylan GB, Rennie JM, Pressler RM, Wilson G, Morton M, Binnie CD: Phenobarbitone, neonatal seizures, and video-EEG. *Arch Dis Child Fetal Neonatal Ed* 2002;86:F165-F170.
- [4] Painter MJ, Scher MS, Stein AD, Armatti S, Wang Z, Gardiner JC, et al: Phenobarbital compared with phenytoin for the treatment of neonatal seizures. *N Engl J Med* 1999;341:485-489.
- [5] Pressler RM, Mangum B: Newly emerging therapies for neonatal seizures. *Semin Fetal Neonatal Med* 2013;18:216-223.
- [6] Jensen FE: Developmental factors in the pathogenesis of neonatal seizures. *J Pediatr Neurol* 2009;7:5-12.
- [7] Malone A, Ryan CA, Fitzgerald A, Burgoyne L, Connolly S, Boylan GB: Interobserver agreement in neonatal seizure identification. *Epilepsia* 2009;50:2097-2101.
- [8] Murray DM, Boylan GB, Ali I, Ryan CA, Murphy BP, Connolly S: Defining the gap between electrographic seizure burden, clinical expression and staff recognition of neonatal seizures. *Arch Dis Child Fetal Neonatal Ed* 2008;93:F187-F191.
- [9] van den Broek MP, Groenendaal F, Toet MC, van Straaten HL, van Hasselt JG, Huitema AD, et al: Pharmacokinetics and clinical efficacy of phenobarbital in asphyxiated newborns treated with hypothermia: a thermopharmacological approach. *Clin Pharmacokinet* 2012;51:671-679.
- [10] Rennie JM, Chorley G, Boylan GB, Pressler R, Nguyen Y, Hooper R: Non-expert use of the cerebral function monitor for neonatal seizure detection. *Arch Dis Child Fetal Neonatal Ed* 2004;89:F37-F40.
- [11] Shellhaas RA, Ng CM, Dillon CH, Barks JD, Bhatt-Mehta V: Population pharmacokinetics of phenobarbital in infants with neonatal encephalopathy treated with therapeutic hypothermia. *Pediatr Crit Care Med* 2013;14:194-202.
- [12] Takemoto CK, Hodding JH, Kraus DM: *The Pediatric Dosage Handbook*, ed 19. Hudson, Ohio, Lexi-Comp, Inc, 2012.
- [13] Klem GH, Luders HO, Jasper HH, Elger C: The ten-twenty electrode system of the International Federation. *The International Federation of Clinical Neurophysiology. Electroencephalogr Clin Neurophysiol Suppl* 1999;52:3-6.
- [14] Clancy RR: Prolonged electroencephalogram monitoring for seizures and their treatment. *Clin Perinatol* 2006;33:649-665.
- [15] Ortibus EL, Sum JM, Hahn JS: Predictive value of EEG for outcome and epilepsy following neonatal seizures. *Electroencephalogr Clin Neurophysiol* 1996;98:175-185.
- [16] Dzhalal VI, Kuchibhotla KV, Glykys JC, Kahle KT, Swiercz WB, Feng G, et al: Progressive NKCC1-dependent neuronal chloride accumulation during neonatal seizures. *J Neurosci* 2010;30:11745-11761.
- [17] Jensen FE: Developmental factors regulating susceptibility to perinatal brain injury and seizures. *Curr Opin Pediatr* 2006;18:628-633.
- [18] Moshe SL: Epileptogenesis and the immature brain. *Epilepsia* 1987;28 Suppl 1:S3-15.
- [19] Jones DM, Esmaeil N, Maren S, Macdonald RL: Characterization of pharmacoresistance to benzodiazepines in the rat Li-pilocarpine model of status epilepticus. *Epilepsy Res* 2002;50:301-312.
- [20] Nardou R, Yamamoto S, Chazal G, Bhar A, Ferrand N, Dulac O, et al: Neuronal chloride accumulation and excitatory GABA underlie aggravation of neonatal epileptiform activities by phenobarbital. *Brain* 2011;134:987-1002.
- [21] Lynch NE, Stevenson NJ, Livingstone V, Murphy BP, Rennie JM, Boylan GB: The temporal evolution of electrographic seizure burden in neonatal hypoxic ischemic encephalopathy. *Epilepsia* 2012;53:549-557.
- [22] Boylan G, Burgoyne L, Moore C, O'Flaherty B, Rennie J: An international survey of EEG use in the neonatal intensive care unit. *Acta Paediatr* 2010;99:1150-1155.
- [23] Boylan GB, Stevenson NJ, Vanhatalo S: Monitoring neonatal seizures. *Semin Fetal Neonatal Med* 2013;18:202-208.

Results

Therapeutic hypothermia was contraindicated in case six who had grade II HIE because there was an extensive subgaleal hematoma and some degree of coagulopathy present. Therapeutic hypothermia was also contraindicated in cases 14 and 16 who were neonates with HIE grade III and II respectively because they presented clinically beyond the therapeutic window (i.e. > six hours of age and as can be seen in Table S1, EEG monitoring was commenced at approximately 17 and 28 hours of age respectively). The doses of phenobarbitone given were at the discretion of the attending neonatologist. Case 11 was the only neonate given a dose of 10 mg/kg as the first dose; it was anticipated that a dose of 20 mg/kg may cause further respiratory depression as there was a history of recurrent apnea when the neonate was not yet intubated and ventilated.

Details on excluded neonates

One of the two neonates who did not receive any ASD had one short seizure (two minutes at 0400 hour) and the other was ventilated and sedated with no clinical seizures noted (electrographic seizure burden: 51 minutes, seven seizure events in total). Five neonates had all their phenobarbitone doses administered shortly after clinical seizures were observed but before EEG monitoring commenced. Eight neonates had phenobarbitone doses administered when there were no ongoing electrographic seizures at that time and one neonate who required cranial imaging did not have EEG monitoring after phenobarbitone doses were administered despite ongoing seizures. One neonate (with seizure burden: 22 minutes, 26 seizure events, mean seizure duration: 51 seconds) had seizures during the rewarming period, but were not noticed for several hours on the weekend; therapeutic hypothermia was recommenced soon after and seizures abated without further ASD administration. Of note, there was a neonate admitted from the postnatal ward who presented with right sided jerking movements for which phenobarbitone was administered [seizure burden: 213 minutes, 70 seizure events, mean seizure duration: 182 seconds; with some recurring electrographic-only seizures (on Friday 0500-1430 hour)]; there were no clinical seizures to alarm the nursing or medical staff. The EEG background pattern was supportive for the diagnosis of HIE but because the presentation was beyond the therapeutic window (beyond six hours), therapeutic hypothermia was not administered. In another neonate who was admitted from the postnatal ward who appeared clinically well with benign familial neonatal seizures (seizure burden: two minutes, four seizure events, mean seizure duration: 28 seconds); some electrographic-only seizures were not noticed during off-call hours (Tuesday 0007 to 0400 hour) when there was no neurophysiologist reporting on-call service and no further ASDs were given after the first dose of phenobarbitone. Two neonates with focal brain lesions who presented with focal seizures had phenobarbitone administered in the postnatal ward before admission to the neonatal intensive care unit for continuous EEG monitoring. One of these two neonates with focal brain lesions had a seizure burden lasting 98 minutes, 36 seizures [with mean seizure duration of 163 seconds; no further clinical signs noted during off-call hours (Saturday 0000 to 1200 hour)] and the other neonate had a seizure burden lasting 142 minutes, nine seizures, mean seizure duration of 944 seconds; ongoing electrographic-only seizures were not noticed during off-call hours (Friday 0230 to 0730 hour, 1030 to 1130 hour, 1400 to 2330 hour and Saturday 0230 to 1400 hour). Seizures were not treated in five neonates after the first dose of phenobarbitone because all seizures were electrographic-only and there were no clinical or aEEG sentinels to alert the clinical team for treatment. The total seizure burden in these five neonates was 477 minutes (145 seizure events, mean seizure duration: 1368 seconds).

Second-line ASDs

Thirteen doses of various second-line ASDs were given to 10 of the 19 neonates with electrographic seizures. The time from the last phenobarbitone dose to the first second-line ASD dose was 5.1 (2.8-6.3) hours. Of these, 12 doses were administered during electrographic seizures in nine neonates (six had phenytoin, three had midazolam). In all 14 excluded neonates who received phenobarbitone, seizures returned despite the administration; seven of whom received second-line ASDs (four had phenytoin, three had midazolam).

Table S1. Characteristics of the 19 neonates with electrographic seizures

| Case | Diagnosis | Cooling duration (age in hours) | Recorded seizure burden (minutes) | Seizure number (n) | Mean seizure duration (seconds) | Age of first ASD | Age at EEG monitoring | Age of first EEG seizure | Duration of EEG monitoring |
|-----------------|--|---------------------------------|-----------------------------------|--------------------|---------------------------------|------------------|-----------------------|--------------------------|----------------------------|
| 1 | Arterial ischemic stroke-LMCA | Non-cooled | 46 | 22 | 126 | 55h 48m | 59h 13m | 59h 43m | 69h |
| 2 ^E | HIE grade II | 72 (2-74) | 58 | 2 | 1741 | 9h 54m | 4h | 8h 10m | 77h 7m |
| 3 | HIE grade II | 72 (2.5-74.5) | 44 | 21 | 127 | 2h 36m | 4h 40m | 11h 23m | 78h 54m |
| 4 ^E | HIE grade II | 72 (2-74) | 119 | 18 | 396 | 15h 36m | 2h 40m | 13h 25m | 101h 15m |
| 5 | Multiple infarctions | Non-cooled | 12 | 4 | 183 | 115h 12m | 113h 0m | 113h 5m | 14h 52m |
| 6 ^E | HIE grade II | Non-cooled | 37 | 1 | 2207 | 7h 18m | 2h 53m | 6h 37m | 60h 55m |
| 7 ^E | HIE grade III | 65 (0.8-66) | 198 | 49 | 243 | 17h 6m | 2h 46m | 16h 33m | 88h 14m |
| 8 | HIE grade III | 91 (2.1-93.1) | 397 | 296 | 80 | 15h 30m | 6h 48m | 11h 47m | 127h 1m |
| 9 | Unknown | Non-cooled | 25 | 8 | 185 | 30h 30m | 32h 57m | 33h 57m | 64h 50m |
| 10 ^E | HIE grade III | 22 (2.5-24.5) | 1404 | 266 | 317 | 9h 18m | 4h 25m | 8h 39m | 172h 22m |
| 11 ^R | HIE grade III | 72 (6-78) | 225 | 198 | 68 | 56h 36m | 4h 50m | 43h 8m | 122h 9m |
| 12 | Arterial ischemic stroke-LMCA and RMCA | Non-cooled | 133 | 22 | 362 | 9h 42m | 9h 4m | 9h 4m | 38h 55m |
| 13 | Multiple infarctions | Non-cooled | 97 | 25 | 234 | 18h 48m | 17h 12m | 28h 13m | 45h 4m |
| 14 ^E | HIE grade III | Non-cooled | 637 | 271 | 141 | 18h 12m | 16h 48m | 16h 50m | 110h 44m |
| 15 ^E | Arterial ischemic stroke-LMCA and LPCA | Non-cooled | 362 | 112 | 194 | 19h | 18h 59m | 18h 59m | 62h 41m |
| 16 | HIE grade II | Non-cooled | 149 | 76 | 117 | 29h | 28h 6m | 28h 20m | 53h 38m |
| 17 ^E | Suspected viral encephalitis | Non-cooled | 80 | 28 | 171 | 57h 30m | 57h 55m | 58h 7m | 103h 56m |
| 18 ^E | Arterial ischemic stroke-RMCA | Non-cooled | 332 | 136 | 146 | 37h 12m | 36h 26m | 36h 26m | 228h 54m |
| 19 | Benign non-familial seizures | Non-cooled | 4 | 5 | 43 | 123h | 119h 25m | 121h | 42h 38m |

ASD: anti-seizure drug; E: neonates with status epilepticus; HIE: hypoxic-ischemic encephalopathy; LMCA: left middle cerebral artery; LPCA: left posterior cerebral artery; R: neonates with electrographic seizures following discontinuation of cooling; RMCA: right middle cerebral artery.

Electroclinical Dissociation of Seizures in Term Neonates

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Short title: Electroclinical dissociation of seizures in neonates

Abbreviations: ECS- electroclinical seizures; ECDS- electroclinical dissociation of seizures; EOS- electrographic-only seizures; EEG- electroencephalogram.

Key words: Anti-seizure medications, electrographic seizures, multichannel EEG, neonates, phenobarbitone, seizure burden.

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Short communications the journal Seizure

Comprise a number of different kinds of previously unpublished materials including short reports or small case series. Short communications should be preceded by an abstract. The body of the text is limited to 1,400 words. There are no more than 12 references, and 2 figures or tables (combined).

Word count: 1400/1400

References: 18

Tables & figures: 2 (combined)

ABSTRACT

An estimate of the incidence of electroclinical dissociation of seizures (ECDS) may be a useful tool to neonatologists in the management of neonatal seizures in term neonates. We aimed to determine the rate of ECDS in term neonates with varying aetiologies. Electrographic seizures were annotated by an experienced neonatal electroencephalographer. Simultaneous video was reviewed in each neonate and the ECDS index was defined as the percentage of accumulated number of electrographic-only seizure (defined as having >40% of their seizure duration with electrographic-only seizures) relative to the total number of seizures in each neonate. Data are expressed as medians (interquartile ranges).

Of 24 neonates with electrographic seizures who had simultaneous video-EEG monitoring, 19 had electrographic seizures and 5 had electroclinical seizures only. Although there was no statistical significance between the groups, the ECDS indices in neonates with focal arterial ischaemic stroke (n=4), neonates with other diagnoses (n=5), cooled [n=7 (3 moderate, 4 severe)] and in non-cooled neonates with HIE [n=3 (2 moderate, 1 severe)] were 64 (58-68)%, 75 (61-89)%, 88 (55-100)% and 94% (n=3) respectively.

Based on our current cohort, the occurrence of ECDS is high. This emphasizes the need for continuous multichannel video EEG monitoring as the majority of electrographic seizures is not detected by clinical observation alone.

(Word count 208)

1. Introduction

The incidence of electroclinical dissociation of seizures (ECDS) has been reported to be as high as 80% of neonates treated with anti-seizure medications.¹⁻⁴ Phenobarbitone may have facilitated the occurrence of ECDS and that different brain pathologies are responsible for the clinical and EEG manifestation of seizures at the molecular level.^{2,4} As the clinical component of ECDS cannot be observed, continuous multichannel video-EEG is crucial in monitoring ECDS in neonates particularly during the treatment period. Neonates with ECDS have higher seizure burden and are associated with poorer neurodevelopmental outcome.⁵ We aimed to unravel the incidence of ECDS in a group of term neonates using continuous multichannel video-EEG.

2. Methods

Institutional review board approval was obtained from the Clinical Research Ethics Committees of Cork Teaching hospitals, Ireland. Written, informed consent was obtained from at least one parent of each neonate who participated in this study. As part of an ongoing study on neonatal seizures at the Cork University Maternity Hospital, Ireland from January 2009 to October 2011, neonates ≥ 37 weeks gestation were enrolled for EEG monitoring if they fulfilled at least two of the following criteria: Apgar score \leq six at five minutes; a continued need for resuscitation after birth; any clinical evidence of encephalopathy or seizures within 72 hours of age. Phenobarbitone was the first-line anti-seizure medication administered to a maximum dose of 40 mg/kg intravenously. Second-line anti-seizure medications were administered if clinical and/or electrographic seizures recurred following phenobarbitone administration. The choice of second-line anti-seizure medication administration was at the discretion of the attending neonatologist.

The method of monitoring based on the multichannel video-EEG using a Nicolet monitor (CareFusion NeuroCare, Wisconsin, USA), the definitions for electrographic seizures, status epilepticus, seizure burden, mean seizure duration and electrographic seizure window have been described in studies previously published by our research group.^{6,7} All electrographic seizures were annotated by an experienced neonatal electroencephalographer (G.B.B). Electrographic-only seizures (EOS) were defined as clear electrographic seizures without any clinical correlates.⁵ Electroclinical seizures were defined as electrographic seizures accompanied with behavioural correlates. The ECDS index was calculated as the percentage of the number of seizures which had $\geq 40\%$ of their seizure duration with EOS; they were calculated relative to the total number of electrographic seizures. Continuous variables were described using medians [interquartile ranges (IQR)] and categorical variables using frequencies. For paired comparisons, the Wilcoxon test was used. All statistical analyses were performed using PASW Statistics 20.0 (IBM SPSS Statistics, Illinois, USA). All tests were two-sided; a p-value < 0.05 was considered to be statistically significant.

3. Results

During the study period, twenty-four neonates with seizures were identified from simultaneous video and multichannel EEG monitoring. The median (IQR) age when EEG monitoring began was 10 (3-19) hours, EEG duration was 68 (47-91) hours and age of first EEG seizure was 19 (13-39) hours. Aetiologies for seizures included hypoxic-ischaemic encephalopathy (HIE) (n=12), stroke (n=6), benign seizures (n=2), intraparenchymal haemorrhage (n=2), subdural haemorrhage (n=1) and cryptogenic seizure (n=1). Table 1

and 2 show details of anti-seizure medications and the characteristics of seizures in each neonate respectively.

Of 24 neonates with electrographic seizures, five neonates (cases 2, 5, 14, 16 and 18) did not have any ECDS; all their seizures were presented as electroclinical seizures only (table 2). In the remaining 19 neonates, 1123 seizures were analyzed. One neonate (case 1) had 44% of seizures identified as EOS while the remaining 18 neonates had $\geq 50\%$ of their total number of seizures detected as EOS [80.03 (54.73-95.01)%]. The median (IQR) ECDS indices in neonates with focal arterial ischaemic stroke (n=4), neonates with other diagnoses (n=5), cooled [n=7 (3 moderate, 4 severe)] and in non-cooled neonates with HIE [n=3 (2 moderate, 1 severe)] were 64 (58-68)%, 75 (61-89)%, 88 (55-100)% and 94% (n=3) respectively.

There was no significant difference in the percentage of the number of seizures with EOS between neonates who received phenobarbitone 20 mg/kg (n=6) vs phenobarbitone 40 mg/kg (n=9) (p=0.516). There was no significant difference in the percentage of the number of seizures with EOS between cooled (n=7) vs all non-cooled neonates (n=12; HIE and other diagnoses) (p=0.498), cooled (n=7) vs non-cooled neonates with HIE (n=3) (p=0.564) and between cooled HIE (n=7) vs non-cooled neonates with focal arterial ischaemic stroke (n=4) (p=0.465).

Also, there was no significant difference in the percentage of the number of seizures with EOS between neonates who had severe HIE (n=5) vs those with moderate HIE (n=5) (p=0.169), between those who had severe HIE (n=5) vs all other diagnoses (n=14) (p=0.711) and between neonates who had status epilepticus (n=8) vs those who had no status epilepticus (n=11) (p=0.804).

4. Discussion

Based on findings of multichannel video-EEG, this study has shown that the ECDS index remains high in term neonates who were treated with anti-seizure medication. The median ECDS indices in neonates with focal arterial ischaemic stroke, other diagnoses, cooled and in non-cooled neonates with HIE were 64, 75, 88 and 94% respectively.

Regional interconnectivity (interhemispheric and corticospinal) which are not fully mature due to incomplete myelination of white matter tracts have been implicated in leading to only modest or no behavioural manifestations of ECDS.⁸ Neonates can show no signs, very subtle tonic or clonic movements, often limited to only one limb, making the diagnosis difficult to discern from myoclonus or other automatisms.^{9,10} The sedative effect of phenobarbitone may account for this, as it is also known to be a potent benzodiazepine. In a cohort of 88% of neonates were treated with anti-seizure medication, up to 79% of neonates had EEG seizures with no clinical correlates.¹¹ ECDS was noted in 58% of neonates with electroclinical seizures after phenobarbitone or phenytoin were administered.^{2,4,12} Anti-seizure medications were administered in 49% of ECDS seizures and 68% of electroclinical seizures, suggesting that anti-seizure medication was not the only factor in causing seizures to dissociate;⁵ other mechanisms in the developing neonatal brain may be responsible for the clinical and EEG seizure manifestation.⁴

There was no significant difference in the ECDS between cooled (n=7) vs non-cooled neonates with HIE (n=7 vs 3) and between cooled vs all non-cooled neonates with various

diagnoses (n=7 vs 12). Using continuous video-EEG (commenced at 10.2 ± 2.9 hours of age for 90.9 ± 28.3 hours) and whole-body cooling in 41 neonates treated with anti-seizure medication (lorazepam, phenobarbitone, fosphenytoin, levetiracetam), Nash *et al.* detected EOS in 34%(14/41) of neonates.¹³ The differing treatment strategies in other institutions using different use of anti-seizure medication may explained the lower incidence of EOS when compared to our study results. The association between ECDS and severe EEG background has been implicated in 6 neonates (25-41 weeks gestation) monitored from 100-360 minutes by Boylan *et al.*,¹ and in 11 neonates by Pinto *et al.*¹⁴ In 11 of 30 neonates with HIE, ECDS was constantly identified only in neonates with depressed and undifferentiated background EEG (defined as EEG activity between 5-15 μ V), implying that ECDS is more common in neonates with severe cerebral injury.¹ Prolonged status epilepticus can cause a progressive state of severe encephalopathy and ECDS has been noted as a feature of prolonged status epilepticus in adults and children.¹⁵⁻¹⁷ Neonatal studies have reported a small incidence of status epilepticus in cohorts of neonates who were cooled. In 47%(8/17) of neonates who had EOS, 23%(4/17) had status epilepticus,¹⁸ while 3/6 with EOS had status epilepticus.¹³ In our study, there were more number of seizures per neonate in the ECDS (n=6) than in the electroclinical group (n=4); $p < 0.05$.

Most electrographic seizures emerged during out-of-hours working time (past midnight and during weekends); there were no alarm systems to alert nursing and medical personnel when there were ongoing electrographic seizures detected on the multichannel video-EEG monitoring device. Hence, treatment of seizures remains suboptimal as many neonates were treated when there were abnormal movements but with no electrographic seizure correlates. Further trials on assessing the effectiveness of treatment would be highly optimized using the automated seizure detection embedded in the EEG system on a continuous monitoring basis, as a method to alert neonatologists to treat when there are ongoing electrographic seizures and not to treat when there are no electrographic seizures.

The high incidence of ECDS raises the important issue of accurate seizure detection if our goal is to optimize neuroprotection in neonates. The findings from this study are important, making it crucial that we develop a more effective method of detecting seizures. Further research should revisit our inevitable reliance on the continuous and prolonged multichannel video-EEG monitoring for seizure surveillance in neonates.

Contributors Geraldine B Boylan developed the hypothesis and conceived the study. Janet M Rennie and Geraldine B Boylan obtained funding for the study. Evonne Low, Nathan J Stevenson, Sean R Mathieson and Geraldine B Boylan compiled and annotated the EEG data, analysed and interpreted the EEG recordings. Vicki Livingstone conducted the statistical analysis. Nathan J Stevenson developed the technical analysis for seizure burden. Evonne Low drafted the manuscript. CA Ryan, Janet M Rennie, Nathan J Stevenson, Vicki Livingstone and Geraldine B Boylan critically reviewed the manuscript and approved the final manuscript as submitted.

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References

1. Boylan GB, Pressler RM, Rennie JM, et al. Outcome of electroclinical, electrographic, and clinical seizures in the newborn infant. *Dev Med Child Neurol* 1999;**41**:819-825.
2. Boylan GB, Rennie JM, Pressler RM, et al. Phenobarbitone, neonatal seizures, and video-EEG. *Arch Dis Child Fetal Neonatal Ed* 2002;**86**:F165-F170.
3. Clancy RR. Prolonged electroencephalogram monitoring for seizures and their treatment. *Clin Perinatol* 2006;**33**:649-665.
4. Scher MS, Alvin J, Gaus L, et al. Uncoupling of EEG-clinical neonatal seizures after antiepileptic drug use. *Pediatr Neurol* 2003;**28**:277-280.
5. Weiner SP, Painter MJ, Geva D, et al. Neonatal seizures: electroclinical dissociation. *Pediatr Neurol* 1991;**7**:363-368.
6. Low E, Boylan GB, Mathieson SR, et al. Cooling and seizure burden in term neonates: an observational study. *Arch Dis Child Fetal Neonatal Ed* 2012;**97**:F267-F272.
7. Low E, Mathieson SR, Stevenson NJ, et al. Early postnatal EEG features of perinatal arterial ischaemic stroke with seizures. *PLoS One* 2014;**9**:e100973.
8. Glykys J, Dzhalal VI, Kuchibhotla KV, et al. Differences in cortical versus subcortical GABAergic signaling: a candidate mechanism of electroclinical uncoupling of neonatal seizures. *Neuron* 2009;**63**:657-672.
9. Mizrahi EM, Kellaway P. *Diagnosis and Management of Neonatal Seizures*. Philadelphia: Lippincott-Raven, 1998.
10. Boylan GB, Stevenson NJ, Vanhatalo S. Monitoring neonatal seizures. *Semin Fetal Neonatal Med* 2013;**18**:202-208.
11. Clancy RR, Legido A. The exact ictal and interictal duration of electroencephalographic neonatal seizures. *Epilepsia* 1987;**28**:537-541.
12. Painter MJ, Scher MS, Stein AD, et al. Phenobarbital compared with phenytoin for the treatment of neonatal seizures. *N Engl J Med* 1999;**341**:485-489.
13. Nash KB, Bonifacio SL, Glass HC, et al. Video-EEG monitoring in newborns with hypoxic-ischemic encephalopathy treated with hypothermia. *Neurology* 2011;**76**:556-562.
14. Pinto LC, Giliberti P. Neonatal seizures: background EEG activity and the electroclinical correlation in full-term neonates with hypoxic-ischemic encephalopathy. Analysis by computer-synchronized long-term polygraphic video-EEG monitoring. *Epileptic Disord* 2001;**3**:125-132.
15. Abend NS, Arndt DH, Carpenter JL, et al. Electrographic seizures in pediatric ICU patients: cohort study of risk factors and mortality. *Neurology* 2013;**81**:383-391.
16. Watanabe K. Neurophysiological aspects of neonatal seizures. *Brain Dev* 2014;**36**:363-371.
17. Abend NS, Wusthoff CJ, Goldberg EM, et al. Electrographic seizures and status epilepticus in critically ill children and neonates with encephalopathy. *Lancet Neurol* 2013;**12**:1170-1179.
18. Wusthoff CJ, Dlugos DJ, Gutierrez-Colina A, et al. Electrographic seizures during therapeutic hypothermia for neonatal hypoxic-ischemic encephalopathy. *J Child Neurol* 2011;**26**:724-728.

Table 1 Details on the sequence of anti-seizure medication given and reasons why ongoing EEG seizures were not treated

| Case | Age of first clinical seizure (hours) | Characteristic of first clinical seizure | Age of EEG monitoring (hours) | Age of first EEG seizure (hours) | Duration of EEG monitoring (hours) | Age of first anti-seizure medication (hours) | Order of anti-seizure medication given during EEG (mg/kg) | Reasons for not given further anti-seizure medication despite ongoing EEG seizures |
|-----------------|---------------------------------------|---|-------------------------------|----------------------------------|------------------------------------|--|---|--|
| 1 | 5 | Myoclonic jerks with desaturations | 1.6 | 13 | 92.28 | 6.2 | PB (20) | Saturday 0200-0900. Sedated with no further clinical signs noted |
| 2 ^E | 6 | Limbs jerking and blinking | 2.9 | 7 | 60.92 | 7.3 | PB (20): non-cooled | Complete resolution of seizures |
| 3 | 11 | Hypertonic limbs with arching | 4.7 | 11 | 78.9 | 11 | PB (20, 10) | Saturday 0200-0300. Sedated with no further clinical signs noted |
| 4 | Not recorded | Fisting and posturing | 12.1 | 22 | 97.08 | None | None: non-cooled | Saturday 0030-0500, Sunday 2300, Monday 0012 |
| 5 ^E | 2 | Upper limbs jerks | 4 | 8 | 77.12 | 9.9 | PB (20) | Sunday 0649-0759. Complete resolution of seizures |
| 6 | 1 | Upper limbs jerks, nystagmus to the right | 9.1 | 19 | 159.65 | 1.1 | PB (20, 10, 10), PT, CL, LV | Given for clinical seizures |
| 7 | 4 | Tremulous right hand | 3.3 | 23 | 84.25 | 7.3 | PB (20) | Monday 0036-0241, 0500-1600. Sedated with no further clinical signs noted |
| 8 ^E | 1 | Generalize tonic clonic after intubation | 2.7 | 13 | 101.25 | 15.6 | PB (20) | Wednesday 1500-1700, 2100 to Thursday 0900. Sedated with no further clinical signs noted |
| 9 ^E | 2 | Right arm jerking | 2.8 | 17 | 88.23 | 17.1 | PB (20, 20), PT | Sedated with no further clinical signs noted |
| 10 ^E | 2 | Right sided limbs jerking | 10.8 | 11 | 49.42 | 10.2 | PB (20): non-cooled | Friday 0500-1430 |
| 11 ^R | 2 | Desaturations while on ventilator | 4.8 | 43 | 122.15 | 56.6 | PB (10,10,20), PT,CL | Sunday 0200 (Valentine's day) to Monday 1000. Sedated with no further clinical signs noted |
| 12 ^E | 1 | Eyes staring | 16.8 | 17 | 110.73 | 18.2 | PB (20, 20): non-cooled | Saturday 2300, Sunday 0200, 0500, 2200 |
| 13 | Day 7 | Limbs jerking | 151.3 | 153 | 33.45 | 150.4 | PB (20): non-cooled | Tuesday 0007-0400; only 2 mins long seizure |
| 14 | Day 4 | Upper limb hypertonic, left eye deviation | 119.4 | 121 | 42.63 | 123 | PB (20), PY: non-cooled | Tuesday 1600-1700 |
| 15 | Not recorded | Desaturations | 6.8 | 7 | 56.53 | 8.6 | PB (20, 10, 10), PT, CL, LV, PY: non-cooled | Saturday 0900-1700 |
| 16 | Day 5 | Left focal seizures | 113 | 113 | 14.87 | 115.2 | PB(20): non-cooled | Friday 1800-2030. Complete resolution of seizures |
| 17 | 13 | Left focal seizures | 17.2 | 28 | 45.07 | 18.8 | PB(20, 20): non-cooled | Tuesday 0000,0100,0200, Wednesday 0830 |
| 18 | 42 | Limbs jerking | 33.9 | 34 | 64.83 | 30.5 | PB (20, 20), PT: non-cooled | Saturday 0730-1700 |
| 19 | 54 | Desaturations only | 59.2 | 60 | 69 | 55.8 | PB(20, 10),PT: non-cooled | Saturday 1530-2200 |
| 20 | 18 | Right focal seizures | 21.1 | 22 | 61.02 | 19.6 | PB(20): non-cooled | Saturday 0000-1200. Sedated with no further clinical signs noted |
| 21 ^E | 8 | Left focal seizures | 17.7 | 18 | 73.27 | 10.3 | PB (20, 10), PT: non-cooled | Thursday 1700 to Wednesday 0600 |
| 22 ^E | 33 | Right sided jerks | 3.3 | 39 | 45.63 | 34.4 | PB(20, 10), PT: non-cooled | Thursday 0600-1700 |
| 23 ^E | 15 | Right upper limb jerks | 17.9 | 19 | 51.82 | 23.7 | PB(20, 20): non-cooled | Friday 0230-0730, 1030-1130, 1400-2330. Saturday 0230-1400 |
| 24 ^E | 18 | Right upper limb jerks | 18.9 | 19 | 62.68 | 19 | PB(20, 10, 10), PT: non-cooled | Thursday 1700-0000. Friday 0000 to Saturday 0400 |

B: neonates who had all phenobarbitone doses given before EEG monitoring commence; N: neonates with no anti-seizure medication given at any stage; *neonates who were given at least one of the phenobarbitone dose during EEG monitoring, but all phenobarbitone doses were not given during EEG seizures.

Table 2 Characteristics of EEG seizures

| Case | Seizure burden (mins) | Number of seizures (n) | Mean seizure duration (seconds) | Seizure window (hours) | Clonic seizures % (n) (description) | Subtle seizures % (n) (description) | Obscured seizures % (n) | Electroclinical seizures % (n) | EEG-only seizures % (n) |
|------|-----------------------|------------------------|---------------------------------|------------------------|-------------------------------------|-------------------------------------|-------------------------|--------------------------------|-------------------------|
| 1 | 24 | 9 | 161 | 9 | 0 (0) | 55.56 (5) (B) | 0 (0) | 55.56 (5) | 44.44 (4) |
| 2 | 37 | 1 | 2207 | 1 | 100 (1) (L-sided) | 0 (0) | 0 (0) | 100 (1) | 0 (0) |
| 3 | 44 | 21 | 127 | 11 | 4.76 (1) (RUL) | 14.29 (3) (B, T) | 0 (0) | 19.05 (4) | 80.95 (17) |
| 4 | 51 | 7 | 438 | 29 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 100 (7) |
| 5 | 58 | 2 | 1741 | 1 | 50 (1) (arching) | 50 (1) (St) | 0 (0) | 100 (2) | 0 (0) |
| 6 | 67 | 89 | 45 | 118 | 1.12 (1) (UL) | 39.33 (35) (B, Bx, Cy, St) | 4.49 (4) | 40.05 (36) | 55.06 (49) |
| 7 | 78 | 54 | 87 | 15 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 100 (54) |
| 8 | 119 | 18 | 396 | 17 | 0 (0) | 5.56 (1) (Hi) | 5.56 (1) | 5.56 (1) | 88.89 (16) |
| 9 | 198 | 49 | 243 | 41 | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 100 (49) |
| 10 | 213 | 70 | 182 | 31 | 1.43 (1) (RUL) | 2.86 (2) (Cr) | 1.43 (1) | 4.29 (3) | 94.29 (66) |
| 11 | 225 | 198 | 68 | 51 | 0 (0) | 0.51 (1) (D) | 7.07 (14) | 0.51 (1) | 92.42 (183) |
| 12 | 637 | 271 | 141 | 92 | 7.38 (20) (arching, UL) | 8.86 (24) (B, D, Hb, Hy, M, Sh, St) | 3.69 (10) | 16.24 (44) | 80.07 (217) |
| 13 | 2 | 4 | 28 | 3 | 25 (1) (LLL) | 0 (0) | 0 (0) | 25 (1) | 75 (3) |
| 14 | 4 | 5 | 43 | 19 | 60 (3) (L-sided) | 20 (1) (M) | 20 (1) | 80 (4) | 0 (0) |
| 15 | 7 | 5 | 81 | 8 | 0 (0) | 0 (0) | 20 (1) | 0 (0) | 80 (4) |
| 16 | 12 | 4 | 183 | 2 | 75 (3) (LUL) | 0 (0) | 25 (1) | 75 (3) | 0 (0) |
| 17 | 18 | 6 | 176 | 8 | 0 (0) | 33.33 (2) (Cy) | 0 (0) | 33.33 (2) | 66.67 (4) |
| 18 | 25 | 8 | 185 | 9 | 100 (8) (RUL) | 0 (0) | 0 (0) | 100 (8) | 0 (0) |
| 19 | 67 | 41 | 98 | 9 | 0 (0) | 24.39 (10) (D) | 9.76 (4) | 24.39 (10) | 65.67 (27) |
| 20 | 98 | 36 | 163 | 12 | 2.78 (1) (LLL) | 0 (0) | 0 (0) | 2.78 (1) | 97.22 (35) |
| 21 | 142 | 9 | 944 | 28 | 55.56 (3) (LUL) | 11.11 (1) (Cl) | 0 (0) | 44.44 (4) | 55.56 (5) |
| 22 | 201 | 23 | 523 | 11 | 0 (0) | 39.13 (9) (M, Su) | 4.35 (1) | 39.13 (9) | 56.52 (13) |
| 23 | 266 | 112 | 143 | 21 | 68.75 (17) (RUL) | 13.39 (15) (M, Su) | 2.68 (3) | 28.57 (32) | 68.75 (77) |
| 24 | 327 | 101 | 195 | 35 | 61.39 (16) (RUL) | 18.81 (19) (Cy, M, Su, Y) | 3.96 (4) | 34.65 (35) | 61.39 (62) |

B: blinking; Bx: boxing; Cl: clenching of fists; Cr: crying; Cy: cycling of limbs; D: desaturations of peripheral oxygen; Electroclinical seizures: electrographic seizures accompanied with behavioural correlates; Electrographic-only seizures: clear electrographic seizures without any clinical correlates; Electrographic seizure window: the timepoint between the first and last recorded electrographic seizure in hours. Hb: head bobbing; Hi: hiccups; Hy: hyperventilating; L: left; LLL: left lower limb; M: mouthing; Obscured seizures: seizures when viewing of the video was obscured (for example during a medical procedure); RUL: right upper limb; Seizure burden: the total duration of recorded electrographic seizures in minutes; Sh: shivering; St: staring; Su: sucking; T: twitching of left upper limb; UL: upper limb; Y: yawning.

Early continuous video electroencephalography in neonatal stroke

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Perinatal stroke is the second most common cause of neonatal seizures, and can result in long-term neurological impairment. Diagnosis is often delayed until after seizure onset, owing to the subtle nature of associated signs. We report the early electroencephalographic (EEG) findings in a female infant with a perinatal infarction, born at 41 weeks 2 days and weighing 3.42kg. Before the onset of seizures, the EEG from 3 hours after delivery demonstrated occasional focal sharp waves over the affected region. After electroclinical seizures, focal sharp waves became more frequent, complex, and of higher amplitude, particularly in 'quiet sleep'. In 'active sleep', sharp waves often disappeared. Diffusion-weighted imaging confirmed the infarct, demonstrating left frontal and parietal diffusion restriction. At 9 months, the infant has had no further seizures, and neurological examination is normal. To our knowledge, this report is the first to describe the EEG findings in perinatal stroke before seizures, and highlights the evolution of characteristic background EEG features.

Perinatal stroke is an important cause of long-term neurological morbidity,¹ and the second most common cause of seizures in the newborn period, accounting for 12 to 18% of all neonatal seizures.^{2,3} Most infants who suffer a stroke are well after delivery, and come to attention when they develop clinical seizure activity.² Therefore, electroencephalography (EEG) is generally performed only after presentation with seizures. EEG can monitor ongoing seizure activity, aid diagnosis, and predict outcome.⁴ The EEG features associated with neonatal stroke have been described only peri- or postictally. The early EEG changes that occur before the onset of clinical seizures in neonatal stroke are unknown. We present the early clinical and EEG findings from 3 hours after delivery in a term infant with a middle cerebral artery infarct who progressed to seizures at 33 hours post delivery. Parental informed consent was obtained for publication of this case report.

CASE REPORT

A female infant was born at 41 weeks 2 days to a 34-year-old mother whose pregnancy had been uneventful. The antenatal fetal heart rate and variability were normal. The infant was delivered by ventouse owing to failure to progress. Meconium was present at delivery, and the airway was intubated and aspirated. Positive-pressure ventilation was required for 2 minutes. The heart rate remained over 100 beats per minute throughout. The Apgar scores were 3 at 1 minute and 6 at 5 minutes. The arterial cord pH was 7.27, with a base excess of -7.6mEq/L and bicarbonate of 19.3mmol/L .

The infant was admitted to the neonatal unit with mild tachypnoea requiring 30% fractional inspired oxygen ($F_i\text{O}_2$) for 6 hours. A chest radiograph was consistent with mild

meconium aspiration, and neurological examination at this time was normal. As part of an ongoing research study, continuous digital video EEG began at 3 hours post delivery.

The infant stabilized quickly, and at 18 hours post-delivery EEG was discontinued. The infant remained slow to feed and mildly irritable. Repeated neurological assessment at 24 hours was normal with no obvious encephalopathy.⁵

At 33 hours post delivery, right upper and lower limb clonic movements and eye flickering were observed for 3 to 4 minutes. Two further clinical seizures occurred over the next 45 minutes. Between clinical seizures, the infant remained responsive with normal tone and pupillary reflexes. A loading dose of 20mg/kg phenobarbital was administered intravenously. Continuous EEG monitoring was recommenced at 34 hours post delivery; this confirmed seizure activity. Intermittent electrographic seizures continued and phenytoin was administered at 48 hours. No further seizures were noted after 54 hours.

Initial investigations, including C-reactive protein, full blood count, blood culture, metabolic screen (lactate, ammonia, serum amino acids, and urinary organic acids), and lumbar puncture, did not reveal a cause for the seizures. Cranial ultrasound performed at 44 hours post delivery revealed no abnormalities.

The Amiel-Tison⁶ neurological assessment was performed on day 3. The examination showed the infant to be lethargic, with right thumb adduction, no resistance to the scarf test in the right upper limb, and slow recoil in both the right upper and lower limbs. The Moro reflex was asymmetrical, with decreased movement in the right upper limb. The Amiel-Tison assessment was repeated on day 7. The infant

was alert, the right thumb inactive but not fixed in adduction, and neither elbow reached the midline on scarf test. The right upper limb continued to have slow recoil, but axial motor activity was normal.

Magnetic resonance imaging of the brain was performed at 68 hours. The T1- and T2-weighted sequences were normal, but diffusion-weighted imaging demonstrated extensive left frontal and parietal diffusion restriction, consistent with acute ischaemia of the anterior trunk of the middle cerebral artery, specifically of the prefrontal, precentral, and central branches (Fig. S1, published online). Repeat magnetic resonance imaging on day 10 demonstrated extensive left middle cerebral artery territory white matter T1 hypointensity and T2 hyperintensity, consistent with infarction. Perisylvian cortical T1 hyperintensity, representing cortical laminar necrosis, was also present.

At 9 months of age, the infant has a typical neurological examination with no residual asymmetry, development is typical, and there have been no further seizures. No aetiology for the infarction has yet been found.

Electroencephalographic findings

Continuous video EEG (NicoletOne ICU Monitor; NeuroCare, Carefusion, Middleton, WI, USA) was initiated at 3 hours post delivery using nine scalp electroencephalographic monitoring electrodes (F3, F4, C3, C4, T3, T4, O1, O2, and Cz). The EEG was initiated as part of an ongoing clinical study (The BiHiVE Study; one of the inclusion criteria for the study is an Apgar score of ≤ 6 at 5min). Electrocardiography and respiration monitoring were performed simultaneously. Visual EEG analysis was performed by a clinical scientist experienced in neonatal EEG and by a clinical neurophysiologist (GB).

The EEG at 3 hours demonstrated continuous background activity in the 50 to 100 μ V range, with poorly developed sleep cycles (Fig. 1). Occasional sharp waves were seen over the left central region in 'active sleep' phases. In 'quiet sleep', intermittent bursts of high-amplitude sharp waves were present over the left hemisphere. Some mild asymmetries were also noted between the right and left hemisphere. These findings were not identifiable on the simultaneous amplitude integrated EEG trace. The EEG was recommenced at 34 hours, after clinical seizures were noted. The background EEG asymmetry had become much more obvious in quiet sleep, with bursts of sharp waves and isolated sharp waves occurring every 3 to 5 seconds over the left hemisphere. In addition, runs of rhythmic theta activity were also present over the left hemisphere. In active sleep, the asymmetry lessened and frequently disappeared (Fig. 2). Electrographic seizures demonstrated a clear left-sided focus over the central region. Seizures began as focal 1 to 2Hz discharges. As the seizure evolved, the discharges often became biphasic or triphasic. During the EEG recording, a total of 24 electrographic seizures were recorded, with a mean duration of 8 minutes 43 seconds (range 1min 26s–23min). On simultaneous video recordings, only two were associated with

What this paper adds

- EEG abnormalities in neonatal stroke present early after birth and evolve.
- EEG abnormalities can change with sleep state.

clinical features: one with lip smacking, and one with a tonic-clonic clinical seizure.

DISCUSSION

To our knowledge, this report is the first to document the early postnatal EEG findings in perinatal stroke before the onset of clinical seizures. Animal models have demonstrated that seizure genesis occurs in both the infarct core and the penumbra after stroke, and that non-seizure epileptiform discharges, such as periodic lateralized epileptiform discharges, are generated in the penumbra.^{7,8} In the female infant we assessed, the electrographic abnormalities occurred ipsilateral to the injury and were spatially consistent with the area of hyperintensity on diffusion-weighted imaging.

The exact time of neurological injury causing neonatal seizures is seldom known. Filan et al.⁹ found that seizures emerged between 18 and 20 hours in neonatal hypoxic-ischaemic encephalopathy, whereas Rafay et al.¹⁰ demonstrated that the mean seizure onset in neonatal stroke (at 27.8h) occurred later than in hypoxic-ischaemic encephalopathy. In the female infant we assessed, the seizures developed clinically at 33 hours post delivery. We cannot be exact in the timing of injury; there was no documented sentinel obstetric event. The cardiocotogram was normal before delivery, and the infant was relatively stable at birth. We know that the injury occurred before the infant was 3 hours old, owing to the established presence of abnormal electrographic spikes at that time. The timing of seizures and the diffusion-weighted imaging changes at 66 hours post delivery indicate that the injury occurred close to the time of delivery.

Previous descriptions of EEG abnormalities in perinatal stroke, based upon EEGs after the onset of seizure activity, relate focal slowing and sharp waves.^{11,12} Clancy et al.¹² also noted localized voltage reduction and focal electrical seizures. In the female infant we assessed, asymmetrical activity, focal sharp waves, and theta activity were noted, before and after the onset of seizures. These abnormalities became more prominent with time after injury. Peri-infarct depolarizations (a form of spreading depression) propagate from the infarct core, causing depolarization within the penumbra, and increase the ischaemic tissue volume.¹³ Animal models have demonstrated that this increase occurs within the first 24 hours, perhaps explaining the evolution of the EEG abnormalities described here.¹³

Rando et al.¹⁴ described periodic lateralized epileptiform discharges on the EEG in two instances of neonatal cerebral infarction. These EEGs demonstrated typical background activity with periods of varying duration consisting of repetitive stereotyped discharges (200ms occurring every 1–2s). The discharges were initially negative biphasic sharp transients with an amplitude of 50 to 150 μ V, and were present in all behavioural states. Similar sharp waves were identified before and after the onset of seizure activity in

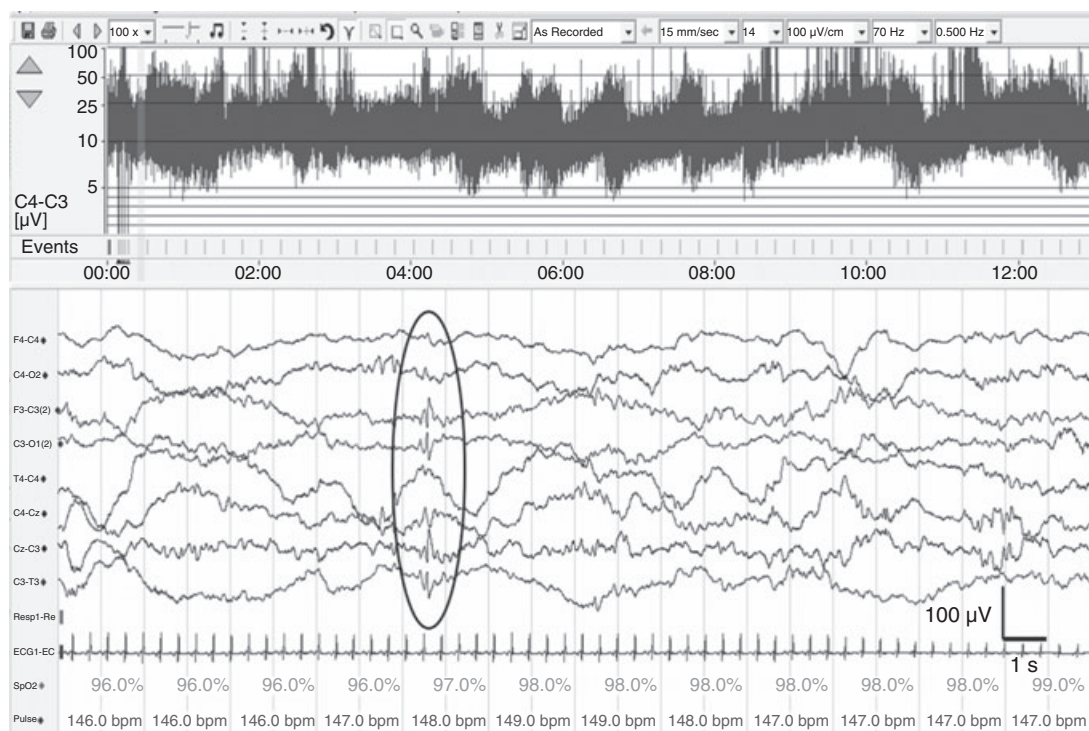


Figure 1: Active sleep before onset of seizures with occasional sharp waves (circled).

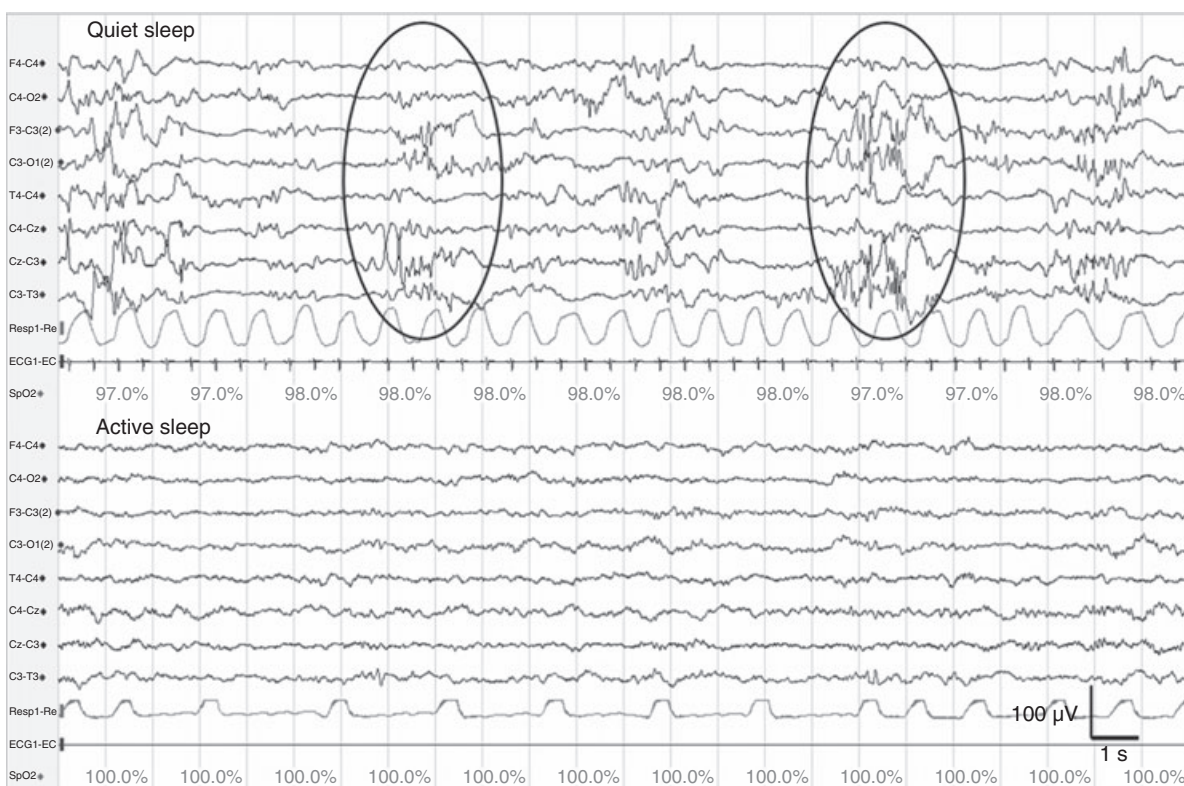


Figure 2: After seizure onset, quiet sleep showing asymmetrical bursts (circled), and active sleep without asymmetry.

our female infant. However, a clear difference in sharp wave activity was noted between active sleep and quiet sleep, being most prominent in quiet sleep. To our knowledge, this has not been previously described in neonatal stroke. Non-rapid eye movement sleep has been shown to increase epileptogenic activity in the animal model.¹⁵ The authors hypothesized that seizure foci were hyperresponsive to synchronous excitatory synaptic inputs, resulting in the propagation of epileptic discharges during non-rapid eye movement sleep.

CONCLUSION

Our findings support the theory that the timing of infarction is during a narrow perinatal window. We have demonstrated that the EEG features previously described in neonatal stroke are present in the early neonatal period before the onset of sei-

zures, and that they progress and become more evident over the first days of life. We also demonstrate that the abnormalities can change with sleep state and disappear during active sleep. This has not been previously described, and highlights the importance of prolonged EEG recording, encompassing at least an entire sleep-wake cycle to ensure that abnormalities are not missed.

ACKNOWLEDGEMENTS

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ONLINE MATERIAL

Additional material and supporting information may be found in the online version of this article..

REFERENCES

1. Sreenan C, Bhargava R, Robertson CMT. Cerebral infarction in the term newborn: clinical presentation and long-term outcome. *J Pediatr* 2000; **137**: 351–5.
2. Estan J, Hope P. Unilateral neonatal cerebral infarction in full term infants. *Arch Dis Child* 1997; **76**: F88–93.
3. Tekgul H, Gauvreau K, Soul J, et al. The current etiologic profile and neurodevelopmental outcome of seizures in term newborn infants. *Pediatrics* 2006; **117**: 1270–80.
4. Nelson KB, Lynch JK. Stroke in newborn infants. *Lancet Neurol* 2004; **3**: 150–8.
5. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress – clinical and electroencephalographic study. *Arch Neurol* 1976; **33**: 696–705.
6. Amiel-Tison C. Update of the Amiel-Tison neurologic assessment for the term neonate or at 40 weeks corrected age. *Pediatr Neurol* 2002; **27**: 196–202.
7. Hartings JA, Williams AJ, Tortella FC. Occurrence of non-convulsive seizures, periodic epileptiform discharges, and intermittent rhythmic delta activity in rat focal ischemia. *Exp Neurol* 2003; **179**: 139–49.
8. Williams AJ, Lu XM, Slusher B, Tortella FC. Electroencephalogram analysis and neuroprotective profile of the N-acetylated-alpha-linked acidic dipeptidase inhibitor, GPI5232, in normal and brain-injured rats. *J Pharmacol Exp Ther* 2001; **299**: 48–57.
9. Filan P, Boylan GB, Chorley G, et al. The relationship between the onset of electrographic seizure activity after birth and the time of cerebral injury in utero. *BJOG* 2005; **112**: 504–7.
10. Rafay MF, Cortez MA, de Veber GA, et al. Predictive value of clinical and EEG features in the diagnosis of stroke and hypoxic ischemic encephalopathy in neonates with seizures. *Stroke* 2009; **40**: 2402–7.
11. Koelfen W, Freund M, Varnholt V. Neonatal stroke involving the middle cerebral-artery in term infants – clinical presentation, EEG and imaging studies, and outcome. *Dev Med Child Neurol* 1995; **37**: 204–12.
12. Clancy R, Malin S, Laraque D, Baumgart S, Younkun D. Focal motor seizures heralding stroke in full-term neonates. *Am J Dis Child* 1985; **139**: 601–6.
13. Hartings JA, Rolli ML, Lu XCM, Tortella FC. Delayed secondary phase of peri-infarct depolarizations after focal cerebral ischemia: relation to infarct growth and neuroprotection. *J Neurosci* 2003; **23**: 11602–10.
14. Rando T, Ricci D, Mercuri E, et al. Periodic lateralized epileptiform discharges (PLEDs) as early indicator of stroke in full-term newborns. *Neuropediatrics* 2000; **31**: 202–5.
15. Shouse MN, Farber PR, Staba RJ. Physiological basis: how NREM sleep components can promote and REM sleep components can suppress seizure discharge propagation. *Clin Neurophysiol* 2000; **111**: S9–18.

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Case Report

EEG Suppression Associated with Apneic Episodes in a Neonate

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We describe the EEG findings from an ex-preterm neonate at term equivalent age who presented with intermittent but prolonged apneic episodes which were presumed to be seizures. A total of 8 apneic episodes were captured (duration 23–376 seconds) during EEG monitoring. The baseline EEG activity was appropriate for corrected gestational age and no electrographic seizure activity was recorded. The average baseline heart rate was 168 beats per minute (bpm) and the baseline oxygen saturation level was in the mid-nineties. Periods of complete EEG suppression lasting 68 and 179 seconds, respectively, were recorded during 2 of these 8 apneic episodes. Both episodes were accompanied by bradycardia less than 70 bpm and oxygen saturation levels of less than 20%. Short but severe episodes of apnea can cause complete EEG suppression in the neonate.

1. Introduction

Despite the frequency with which apnea occurs in the neonate and the concern about adverse long-term effects [1], few studies have examined the effects of apneic episodes simultaneously with recorded multichannel electroencephalography (EEG) [2]. Previous EEG studies in term neonates presenting with apnea but without an accompanying bradycardia have shown that seizures [3], particularly temporal lobe seizures [4], are a common etiology. The EEG changes associated with non-seizure apneic episodes in term neonates have not been described in detail. One study from 1969 has shown that apneic events during weaning from the ventilator in preterm neonates induced EEG suppression ($<10\ \mu\text{V}$) when oxygen partial pressures fell to approximately 20 mmHg [5]. Whether EEG suppression is a common occurrence during intermittent apneic episodes in neonates is not known and neither is the effect of the duration and severity of these events.

Sustained EEG suppression in the term neonate is a worrying sign and is often seen following the acute phase of moderate to severe hypoxic-ischemic encephalopathy [6]. EEG recovery can take hours or even days, depending on the severity of the primary injury and in very severe

cases, the EEG may only recover with very low amplitude activity. In this case report, we were particularly interested in documenting the EEG changes which occur during intermittent episodes of hypoxia and bradycardia due to apnea in an ex-preterm neonate at term equivalent age.

2. Case Report

A female neonate was delivered by emergency Caesarean-section for maternal hypertension at 32 weeks (birthweight 1.9 kg (75th percentile)). At corrected gestational age of 38 weeks, she presented with apneic events associated with bradycardia and cyanosis. While being mechanically ventilated, she displayed some abnormal movements: hyperextension of the arms, jerking movements of all four limbs, thumb abduction, and hyperextension of the trunk during these apneic events. Prior to EEG monitoring, the neonate received intravenous phenobarbitone (10 mg/kg) and phenytoin (15 mg/kg) when clinical suspicion of seizures was raised. Cranial ultrasound imaging was normal. Chest radiograph showed right middle lobe consolidation secondary to viral bronchiolitis. The apneic events were attributed to intermittent mechanical obstruction of the endotracheal tube by copious secretions relating to bronchiolitis.

TABLE 1: Physiological characteristics of apneic events not associated with complete EEG suppressions and those with complete EEG suppressions recorded in the neonate.

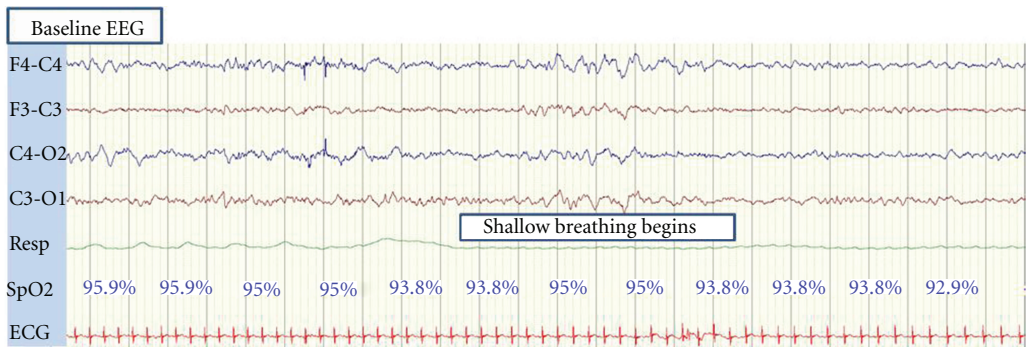
| | Mean (range) | |
|---|--|--|
| | Apnea without complete EEG suppression | Apnea with complete EEG suppression |
| In relation to apneic episodes | | |
| Number of apneic episodes (<i>n</i>) | 6 | 2 |
| Duration of apneic episodes (seconds) | 79 (23–119) | First episode = 213 Second episode = 376 |
| In relation to oxygenation desaturation | | |
| Lowest oxygen desaturation (%) | 45 | First and second episodes <20 (down to unrecordable levels) |
| Duration of oxygen desaturation (seconds) | 137 (72–335) | First episode = 285 Second episode = 361 |
| Lowest oxygen desaturation before complete EEG suppression (%) | — | First episode = 19 Second episode = 4 |
| Duration of oxygen desaturation before complete EEG suppression (seconds) | — | First episode = 55 Second episode = 56 |
| In relation to bradycardia | | |
| Lowest bradycardia (beats per minute) | 99 | First episode = 66 Second episode = 48 |
| Duration of bradycardia (seconds) | 96 (53–224) | First episode = 297 Second episode = 305 |
| Lowest bradycardia before complete EEG suppression (beats per minute) | — | First episode = 66 Second episode = 54 |
| Duration of bradycardia before complete EEG suppression (seconds) | — | First episode = 94 Second episode = 68 |
| In relation to complete EEG suppression | | |
| Number of complete EEG suppression (<i>n</i>) | — | 2 |
| Duration of complete EEG suppression (seconds) | — | First episode = 68 Second episode = 179 |
| Recovery time from oxygen desaturation after complete EEG suppression ended (seconds) | — | First episode = 51 Second episode = 129 |

A NicOne digital video-EEG system (CareFusion NeuroCare, WI, USA) was used to record multichannel EEG in this neonate for a total of 22 hours, using scalp electrodes (F3, F4, C3, C4, T3, T4, O1, O2, and Cz). Continuous vital signs such as respiration, electrocardiogram (ECG), and oxygen saturations were monitored simultaneously using the IntelliVue MP70 Neonatal monitor (Philips, Boeblingen, Germany). The entire EEG recording was reviewed and annotated by an experienced neonatal neurophysiologist (GB). Apnea was defined as cessation of airflow for more than 20 seconds, or cessation of airflow for less than 20 seconds with bradycardia (20% below the baseline heart rate), or cessation of airflow for less than 20 seconds with oxygen desaturations below 80% [7]. Suppression of EEG activity to below 5 μ V in all EEG channels for at least 10 seconds was defined as complete EEG suppression.

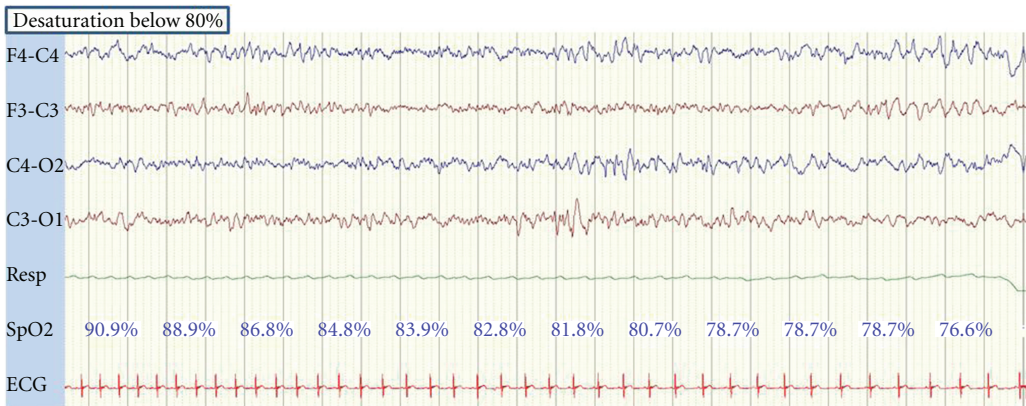
Prior to gestational age of 38 weeks, the neonate did not have any apneic events. The background EEG activity prior to the apneic episodes showed continuous mixed frequency activity with the baseline EEG voltage ranging from 50 to 100 microvolts, which was appropriate for gestational age and electrographic seizure activity was not present before,

during, or after the apneic events. The average baseline heart rate was 168 beats per minute (bpm), oxygenation saturations were in the mid-nineties and the neonate remained normotensive throughout monitoring.

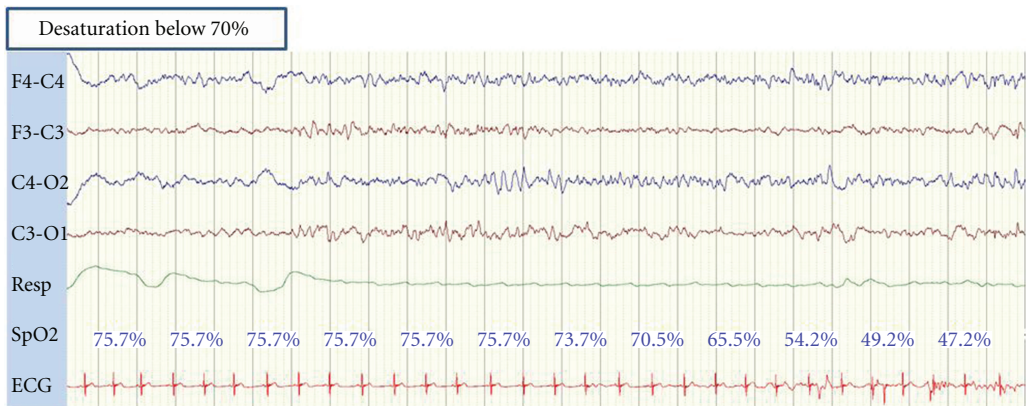
The neonate had a total of eight apneic episodes during EEG monitoring, two of which required intermittent positive pressure ventilation, chest compressions, and adrenaline for recovery. Soon after the onset of both of these more prolonged apneic episodes (duration: 213 and 376 seconds resp.), there was a rapid decline in heart rate to 66 and 54 bpm, respectively, and oxygen saturation decreased to below 20% during both episodes which were accompanied with profound central cyanosis (Table 1). As heart rate and saturations declined, the EEG developed a burst suppression pattern. When the heart rate reached 66 and 54 bpm, respectively, and when oxygen saturations were below 20%, the EEG became completely suppressed. In both of these episodes, the EEG amplitude was completely suppressed for 68 and 179 seconds, respectively. During the recovery phase in both episodes, oxygen saturation improved to approximately 30 to 40% before EEG activity returned. Figures 1(a)–1(j) illustrate the sequence of events associated



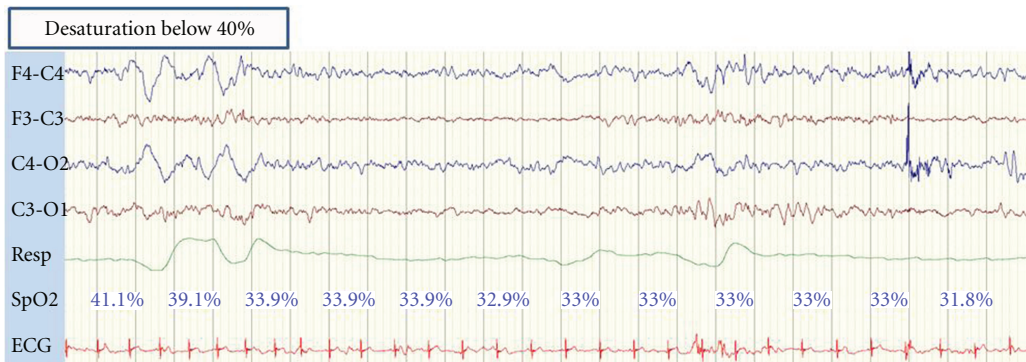
(a)



(b)

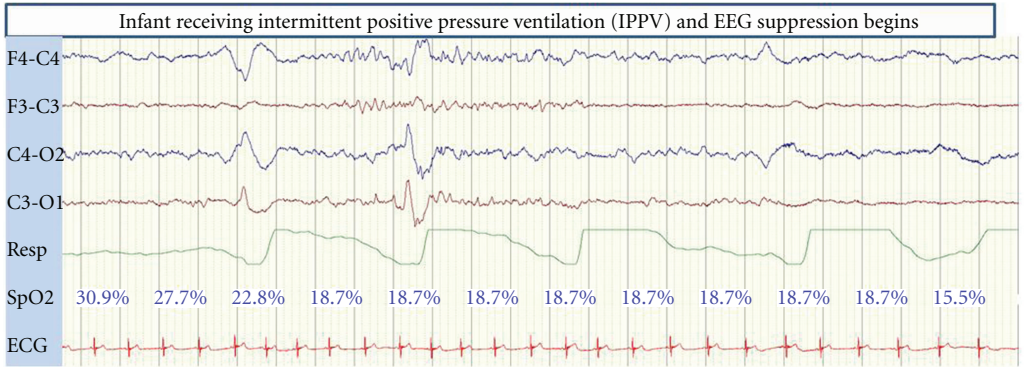


(c)

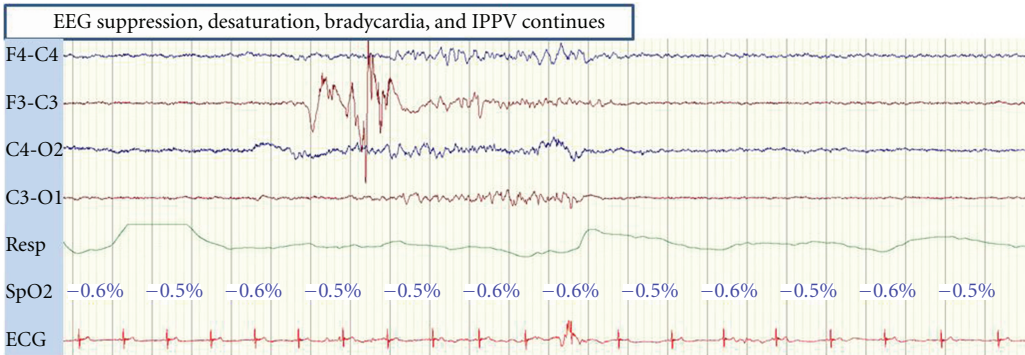


(d)

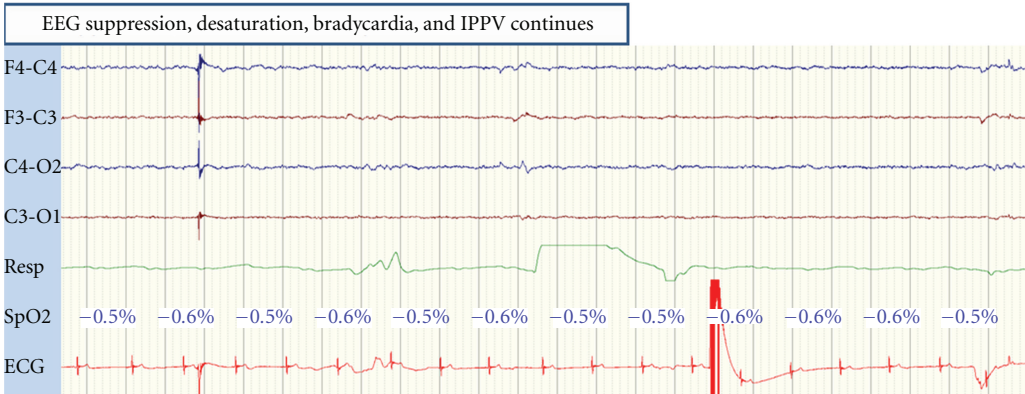
FIGURE 1: Continued.



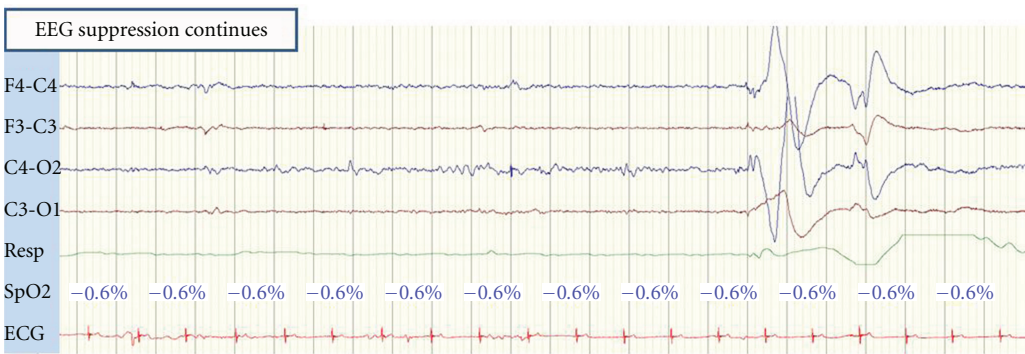
(e)



(f)



(g)



(h)

FIGURE 1: Continued.

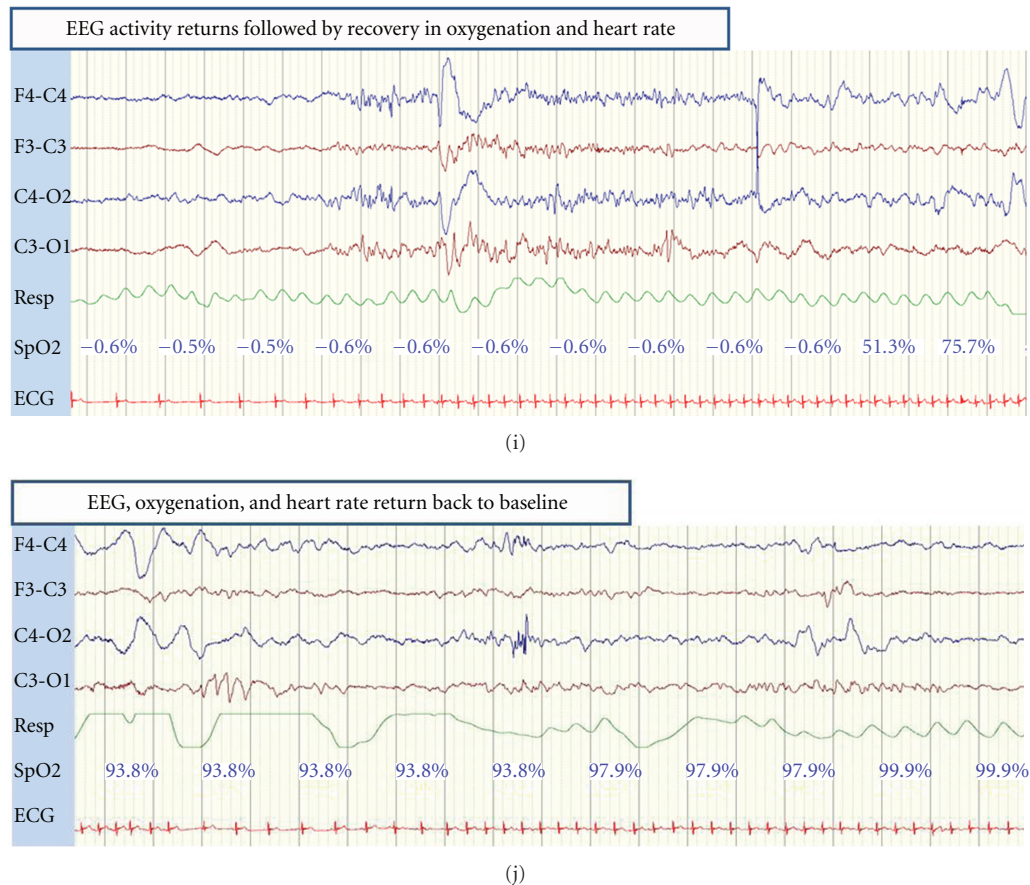


FIGURE 1: EEG recording showing the sequence of events evolving from baseline values associated with the first episode of complete EEG suppression. Calibration is 1 second and 50 microvolts.

with the first episode of complete EEG suppression. The other six recorded apneic episodes in this neonate were less profound in duration (mean (range) = 79 (23–119) seconds) and were not accompanied by any EEG changes. The mean (range) of the lowest oxygen desaturation was 45 (24–69)% and the mean (range) of the lowest bradycardia was 99 (72–132) bpm.

3. Discussion

This case report has shown that episodes of transient but complete EEG suppression can occur during prolonged apneic episodes in the neonate particularly when they are accompanied by profound bradycardia and oxygen desaturation.

The apneic events in our case report were not associated with seizures on the EEG. Although apneic seizures originating in the temporal lobe have been observed in term neonates [4], they are not usually associated with changes in heart rate [3]. In fetal lambs, Gunn et al. has shown that during an ischemic event, the EEG becomes isoelectric [8]. Recovery of EEG activity depended on the duration of the ischemic event, with shorter duration events leading to full recovery of EEG activity. If ischemia lasted 30 minutes or longer, a stereotypic sequence of depressed EEG activity followed

by low frequency epileptiform activity was always seen. In the newborn piglet model, hypoxic-ischemia induced by reducing fractional inspired oxygen to around 6%, led to rapid suppression of EEG activity. Brain damage was only seen when the EEG amplitude remained suppressed for 23 minutes or more [9]. In another study which exposed one-week-old piglets to graded hypoxia, the EEG amplitude did not decline until oxygen saturation fell below 25%, a similar level at which EEG suppression developed in our neonate [10].

In both episodes in our neonate, bradycardia preceded complete EEG suppression and EEG amplitude did not become profoundly suppressed until oxygen saturation fell below 20%. This is similar to the effects described in animal studies when hypoxia has been used to induce severe EEG suppression [11]. In piglets, EEG amplitude has been shown to decrease markedly after approximately 30 seconds of apnea induced by stimulation of the superior laryngeal nerves [11]. Piglets that were preoxygenated preserved their EEG amplitude during stimulation until the oxygen saturation levels fell below 50%. We believe that hypoxia in conjunction with bradycardia was responsible for the severe EEG suppression in our reported case.

Gavilanes et al. have shown that cerebral neuronal oxygenation is maintained during hypoxia-induced EEG

suppression when blood pressure is maintained constantly above 40 mmHg [10]. This suggests that periods of complete EEG suppression during hypoxia may be a neuroprotective mechanism. Animal studies have shown that as soon as cerebral oxygen supply is depleted to a certain critical level, postsynaptic potentials are inhibited by an increase in adenosine (often measured as the breakdown product of hypoxanthine) in the interstitial space via the A1 receptor subtype, resulting in suppression of electrocortical activity [12]. In addition, adenosine may further depress calcium conductance. The actions of adenosine on potassium and calcium metabolism may render the cell less electrically excitable and spare cell energy, avoiding metabolic failure and irreversible cell damage [13]. In rats, immature neurons have been found to be more resistant than adult neurons exposed to hypoxic events. The mechanism for this may be mediated by activation of the N-methyl-D-aspartate receptors or intracellular calcium in the immature brain [13, 14].

Short periods of fetal electrocortical suppression have been reported during labor in humans without any consequences [15]. An adaptive mechanism has been implicated in such short suppression of synaptic transmission activity, where a state of decrease energy requirement is developed to withstand longer hypoxic insults induced by episodes of complete cord occlusion (to mimic uterine contraction in labour) in animal models [16]. In an ovine fetal brain, this adaptive metabolic shutdown appears to be mediated also by endogenous activation of adenosine A1 receptors during critical decreases in oxygenation [17, 18]. The onset of this response has been shown to occur within 30 to 60 seconds after complete cord occlusion in animal models, as measured by a decreased in EEG amplitude or cerebral metabolic rate [17, 18].

Using near-infrared spectrometry, a combination of bradycardia and hypoxia has been shown to impair cerebral oxygenation in the human neonate [19], and this may have a role in the pathogenesis of neonatal cerebral injury. Postnatally, it is not known how long apnea or hypoxia can continue before irreparable brain damage occurs. However, it is known that prolonged suppression of electrocortical activity in the neonate is an ominous sign such as that seen following a severe hypoxic-ischemic brain injury. EEG activity may recover but a long recovery period following hypoxic-ischemic injury is associated with an unfavourable long-term neurological outcome [6]. In animal models, EEG suppression following a severe hypoxic-ischemic insult can occur very rapidly and the time required for recovery will depend on the duration and severity of the primary insult [20].

4. Conclusion

Our case report has shown that prolonged apneic episodes accompanied by hypoxia and bradycardia can be associated with altered cerebral function in the neonate. From a clinical perspective, we feel that clinicians would be keen to know the lowest limit of oxygen saturation required to suppress EEG activity. We have shown that not all apneic

events are associated with complete EEG suppression, but apneic events with oxygen desaturations below 20% always were. Although complete EEG suppression can be reversible, clinicians should be aware that the recovery from complete EEG suppression depends on the speed of intervention. We have shown that EEG amplitude is exquisitely sensitive to hypoxia and bradycardia in the human neonate.

Conflict of Interests

The authors declare no conflict of interests.

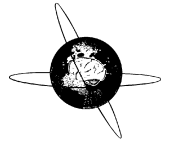
Acknowledgments

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References

- [1] A. Janvier, M. Khairy, A. Kokkoti, C. Cormier, D. Messmer, and K. J. Barrington, "Apnea is associated with neurodevelopmental impairment in very low birth weight infants," *Journal of Perinatology*, vol. 24, no. 12, pp. 763–768, 2004.
- [2] K. Paul, J. Melichar, J. Miletin, and J. Dittrichová, "Differential diagnosis of apneas in preterm infants," *European Journal of Pediatrics*, vol. 168, no. 2, pp. 195–201, 2009.
- [3] G. M. Fenichel, B. J. Olson, and J. E. Fitzpatrick, "Heart rate changes in convulsive and nonconvulsive neonatal apnea," *Annals of Neurology*, vol. 7, no. 6, pp. 577–582, 1980.
- [4] D. Sirsi, L. Nadiminti, M. A. Packard, M. Engel, and G. E. Solomon, "Apneic seizures: a sign of temporal lobe hemorrhage in full-term neonates," *Pediatric Neurology*, vol. 37, no. 5, pp. 366–370, 2007.
- [5] N. R. Robertson, "Effect of acute hypoxia on blood pressure and electroencephalogram of newborn babies," *Archives of Disease in Childhood*, vol. 44, no. 238, pp. 719–725, 1969.
- [6] D. M. Murray, G. B. Boylan, C. A. Ryan, and S. Connolly, "Early EEG findings in hypoxic-ischemic encephalopathy predict outcomes at 2 years," *Pediatrics*, vol. 124, no. 3, pp. e459–e467, 2009.
- [7] D. J. Henderson-Smart, A. G. Pettigrew, and D. J. Campbell, "Clinical apnea and brain-stem neural function in preterm infants," *The New England Journal of Medicine*, vol. 308, no. 7, pp. 353–357, 1983.
- [8] A. J. Gunn, J. T. Parer, E. C. Mallard, C. E. Williams, and P. D. Gluckman, "Cerebral histologic and electrocorticographic changes after asphyxia in fetal sheep," *Pediatric Research*, vol. 31, no. 5, pp. 486–491, 1992.
- [9] M. Thoresen, K. Haaland, E. M. Løberg et al., "A piglet survival model of posthypoxic encephalopathy," *Pediatric Research*, vol. 40, no. 5, pp. 738–748, 1996.
- [10] A. W. D. Gavilanes, J. S. H. Vles, K. Von Siebenthal, R. Van Sprundel, J. P. H. Reulen, and C. E. Blanco, "Neonatal electrocortical brain activity and cerebral tissue oxygenation during non-acidotic, normocarbic and normotensive graded hypoxemia," *Clinical Neurophysiology*, vol. 115, no. 2, pp. 282–288, 2004.
- [11] U. M. Sanocka, D. F. Donnelly, and G. G. Haddad, "Cardiovascular and neurophysiologic changes during graded duration

- of apnea in piglets,” *Pediatric Research*, vol. 23, no. 4, pp. 402–407, 1988.
- [12] A. Ilie, D. Ciocan, A. M. Zagrean, D. A. Nita, L. Zagrean, and M. Moldovan, “Endogenous activation of adenosine A1 receptors accelerates ischemic suppression of spontaneous electrocortical activity,” *Journal of Neurophysiology*, vol. 96, no. 5, pp. 2809–2814, 2006.
- [13] M. Nabetani, Y. Okada, S. Kawai, and H. Nakamura, “Neural activity and the levels of high energy phosphates during deprivation of oxygen and/or glucose in hippocampal slices of immature and adult rats,” *International Journal of Developmental Neuroscience*, vol. 13, no. 1, pp. 3–12, 1995.
- [14] H. J. Luhmann and T. Kral, “Hypoxia-induced dysfunction in developing rat neocortex,” *Journal of Neurophysiology*, vol. 78, no. 3, pp. 1212–1221, 1997.
- [15] M. G. Rosen, J. J. Scibetta, and C. J. Hochberg, “Fetal electroencephalography. IV. The FEEG during spontaneous and forceps births,” *Obstetrics and Gynecology*, vol. 42, no. 2, pp. 283–289, 1973.
- [16] M. G. Frasch, A. E. Keen, R. Gagnon, M. G. Ross, and B. S. Richardson, “Monitoring fetal electrocortical activity during labour for predicting worsening acidemia: a prospective study in the ovine fetus near term,” *PLoS ONE*, vol. 6, no. 7, article e22100, 2011.
- [17] C. J. Hunter, L. Bennet, G. G. Power et al., “Key neuroprotective role for endogenous adenosine A1 receptor activation during asphyxia in the fetal sheep,” *Stroke*, vol. 34, no. 9, pp. 2240–2245, 2003.
- [18] M. Kaneko, S. White, J. Homan, and B. S. Richardson, “Cerebral blood flow and metabolism in relation to electrocortical activity with severe umbilical cord occlusion in the near-term ovine fetus,” *American Journal of Obstetrics and Gynecology*, vol. 188, no. 4, pp. 961–972, 2003.
- [19] L. N. Livera, S. A. Spencer, M. S. Thorniley, Y. A. B. D. Wickramasinghe, and P. Rolfe, “Effects of hypoxaemia and bradycardia on neonatal cerebral haemodynamics,” *Archives of Disease in Childhood*, vol. 66, no. 4, pp. 376–380, 1991.
- [20] C. E. Williams, A. J. Gunn, C. Mallard, and P. D. Gluckman, “Outcome after ischemia in the developing sheep brain: an electroencephalographic and histological study,” *Annals of Neurology*, vol. 31, no. 1, pp. 14–21, 1992.



Validation of an automated seizure detection algorithm for term neonates

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HIGHLIGHTS

- Seizure detection algorithm (SDA) validated on unseen, unedited EEG of 70 neonates.
- Results at SDA sensitivity settings of 0.5–0.3 acceptable for clinical use.
- Seizure detection rate of 52.6–75.0%, false detection rate 0.04–0.36 FD/h.

ABSTRACT

Objective: The objective of this study was to validate the performance of a seizure detection algorithm (SDA) developed by our group, on previously unseen, prolonged, unedited EEG recordings from 70 babies from 2 centres.

Methods: EEGs of 70 babies (35 seizure, 35 non-seizure) were annotated for seizures by experts as the gold standard. The SDA was tested on the EEGs at a range of sensitivity settings. Annotations from the expert and SDA were compared using event and epoch based metrics. The effect of seizure duration on SDA performance was also analysed.

Results: Between sensitivity settings of 0.5 and 0.3, the algorithm achieved seizure detection rates of 52.6–75.0%, with false detection (FD) rates of 0.04–0.36 FD/h for event based analysis, which was deemed to be acceptable in a clinical environment. Time based comparison of expert and SDA annotations using Cohen's Kappa Index revealed a best performing SDA threshold of 0.4 (Kappa 0.630). The SDA showed improved detection performance with longer seizures.

Conclusion: The SDA achieved promising performance and warrants further testing in a live clinical evaluation.

Significance: The SDA has the potential to improve seizure detection and provide a robust tool for comparing treatment regimens.

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1. Introduction

The concept of “neuroprotective” intensive care has now reached neonatal units worldwide, in part driven by the results of randomized controlled trials showing that therapeutic hypothermia is beneficial for term babies with a recent hypoxic-ischaemic injury (Glass et al., 2011). The practice of neuroprotective care involves careful monitoring of carbon dioxide tension, blood pressure and other physiological variables and is ideally accompanied by continuous cotside EEG monitoring. Without EEG

monitoring many seizures are missed. The inaccuracy of clinical recognition of seizures was demonstrated by Murray et al. (2008). In this study, comparing EEG evidence of seizures to the seizure detection acumen of NICU staff based on clinical evidence alone, of 526 EEG seizures, only 179 (34%) had any clinical accompaniment, overdiagnosis was common with only 48 of 177 (27%) clinically suspected events accompanied by EEG seizures such that only 48/526 (9%) of EEG seizures were correctly identified by clinical observation. Amplitude-integrated EEG (aEEG) is widely used in NICUs for seizure detection but has been shown to perform poorly (Rennie et al., 2004). In this study seizure detection by four non-experts using CFM traces at 3 paper speeds were compared against simultaneous EEG in 19 babies. Sensitivities of only 38%

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at 6 cm/h, 54% at 15 cm/h and 55% at 30 cm/h were achieved and agreement between observers was poor at all speeds (κ values from 0.01 to 0.39). Treating seizures to electrical quiescence has yet to be proven of any long-term benefit, but there is evidence from animal models (Wirrell et al., 2001), and clinical studies (Glass et al., 2009; Shah et al., 2014) which would support the principle that seizures do inflict further brain injury. Attempts to ameliorate such damage must be accompanied by prompt and reliable detection of seizures. In addition, good quality randomized controlled trials of new antiepileptic drugs are impossible without robust and reproducible EEG monitoring.

A significant barrier to the practice of neuroprotective critical care in the NICU is the lack of expertise in reporting neonatal EEG. Current cotside EEG monitors are sophisticated devices, offering the ability to record multiple channels of EEG continuously together with other physiological signals and video-recording of the baby's movements. They allow the continuous display of aEEG and other quantitative trends and are easy to set up and maintain. But few clinicians have the knowledge to interpret the plethora of information which is generated by such monitoring, and without this knowledge there is a danger that this equipment will be under utilised or (worse) the output will be misinterpreted at the cotside.

Our group has considerable experience with cotside EEG monitoring and has grown to appreciate the benefits that this provides. For many years now we have been working on a seizure detection algorithm (SDA), which would analyse one or more channels of “raw” EEG, continuously and in real-time, providing a visual and audible alert to the clinical team. The engineering challenges have proven formidable because EEG is a complex signal, and neonatal seizures have variable amplitude, frequency and morphology, and are rarely sustained for more than 5 min.

Other groups have developed SDAs for neonates, and have published their detection rates, using varying definitions of success (Liu et al., 1992; Gotman et al., 1997; Smit et al., 2004; Navakatikyan et al., 2006; Deburchgraeve et al., 2008; Mitra et al., 2009). Details of the performance of these and other SDAs are outlined in Table 1 and reviewed further in the discussion. Currently only two SDAs are commercially available. These are the Gotman algorithm incorporated into the Stellate EEG system (Natus Medical Inc, USA); and the ‘Recognize’ algorithm of Navakatikyan which is incorporated into the Brainz aEEG monitor (Natus Medical Inc., USA) which has only a 2 channel EEG capability. One problem which inhibits comparison of SDAs is the lack of an agreed definition of what constitutes best performance. Many SDAs are reported to have good detection rates, with a high number of seizures accurately detected when compared to expert neurophysiology as the “gold standard”, and low numbers of missed seizures. However, the temporal aspect of seizure detection is rarely reported, for example one missed seizure of 8 min duration in an hour would be clinically important. Another important aspect

of SDA performance assessment is the number of false detections. Many validation studies have used only short duration recordings, but any robust algorithm designed for current NICU use has to be able to perform reliably on very long recordings of 72 h or more. Respiration artefact is a particular problem often recorded in neonatal EEG and can mimic the stereotyped rhythmic seizure activity that is often seen in neonates.

We have previously reported the performance of our neonatal SDA on a set of 17 seizure babies recorded at Cork University Maternity Hospital (CUMH), Ireland (Temko et al., 2011a) using a ‘leave one out’ (LOO) cross validation method of analysis, whereby the data of one patient is used for testing and the others used for training the algorithm and the process is repeated for each patient and the mean result reported. A further LOO study was performed on 38 babies from CUMH (Temko et al., 2013) incorporating an adaptation to reduce the effects of prolonged artefact and showed improved performance. This study also incorporated analysis of an ‘unseen’ dataset of 51 babies from CUMH.

The aim of the present study was to validate the performance of our neonatal SDA on a larger database of unseen, unedited, continuous, multi-channel EEG data from 70 term newborns collected at 2 sites, CUMH and University College London Hospital (UCLH), and to provide comprehensive measures of SDA performance. While time based metrics assess the ability of the algorithm to detect the ‘amount’ of seizure activity (seizure burden) correctly and is, in a sense, the most precise engineering metric, event based metrics provide clinicians with valuable information as to the percentage of seizures that will be detected, with important implications for treatment and also how often the algorithm is likely to alarm falsely. We therefore report both time based and event based measures of performance.

2. Methods

2.1. Data acquisition and EEG annotation

Neonates were enrolled from the neonatal intensive care units of CUMH and UCLH from January 2009 to October 2011 as part of an on-going study of neonatal seizures. Neonates ≥ 37 weeks gestation were enrolled for EEG monitoring if they fulfilled two or more of the following criteria: Apgar score less than six at five minutes; a continued need for resuscitation after birth; any clinical evidence of encephalopathy, or seizures developed within 72 h of age.

This study was conducted with approval from the Clinical Research Ethics Committees of the Cork Teaching hospitals, Ireland and the National Health Service in the UK, via the Integrated Research Application Service. Written, informed consent was obtained from at least one parent of each neonate who participated in this study.

Table 1
Summary of SDAs proposed in the literature. (DB – database, h – hour, S – seizure, NS – non-seizure, Dur – duration, AUC – area under the receiver operator characteristic, Sens – sensitivity, spec, specificity, SDR – seizure detection rate, FA/h – false alarms per hour).

| Algorithm | DB length h (N) | S:NS Dur | NS neonates | AUC | Sens (%) | Spec (%) | SDR (%) | FA/h (N/h) |
|-----------------------------|-----------------|----------|-------------|------|----------|----------|---------|------------|
| Liu et al. (1992) | 1.0 (14) | 1:1 | Yes | | 84 | 98 | | |
| Gotman et al. (1997) | 237 (54) | | Yes | | | | 66 | 2.3 |
| Smit et al. (2004) | 10.4 (19) | | No | | 66 | 90 | | |
| Navakatikyan et al. (2006) | 24 (55) | 1:6.8 | Yes | | 83 | 87 | 90 | 2 |
| Lawrence et al. (2009) | 2708 (40) | | Yes | | | | 55 | 0.09 |
| Deburchgraeve et al. (2008) | 218 (26) | | Yes | | | | 85 | 0.66 |
| Cherian et al. (2011) | 756 (24) | 1:27.9 | No | | 59 | | 66 | 0.58 |
| Mitra et al. (2009) | 120 (76) | 1:11.0 | Yes | | | | 80 | 0.78 |
| Temko et al. (2011a,b) | 268 (17) | 1:5.9 | No | 0.96 | 90 | 90 | 89 | 1 |
| Temko et al. (2013) | 2540 (51) | | Yes | 0.96 | | | 71 | 0.25 |

2.1.1. EEG recording

The EEG was recorded using a NicoletOne EEG monitor (Carefusion, Wisconsin, USA) and the 10:20 EEG electrode placement system adapted for neonates was used with the following electrodes F4, F3, T4, T3, C4, C3, CZ, O2 and O1. Additional electrodes were positioned at P3 and P4 when possible. Respiration and ECG was also monitored and signals were stored synchronously with the EEG. The EEG was recorded at a sampling rate of 250 Hz or 256 Hz, with a filter bandwidth of 0.5–70 Hz. The EEG was recorded from as soon as possible after birth and the recording continued for as long as clinically required.

2.1.2. EEG analysis

All seizures were annotated on the original EEG file by a trained electrophysiologist, Sean Mathieson (SM) to generate seizure event text files for each recording. The seizure annotations of SM for all neonates were used for comparison with the SDA annotations. To verify the validity of the seizure annotations by SM, a random sample of 15/35 (42.85%) recordings with seizures were also annotated by Geraldine Boylan (GB) and compared for inter-rater reliability using Cohen's Kappa index.

An electrographic seizure was defined as a sudden and evolving repetitive stereotyped waveform with a definite start, middle and end, lasting for at least 10 s on at least one EEG channel (Clancy and Legido, 1987). A stand alone, offline version of the SDA was then used to process each EEG recording (see Fig. 1). Full details of the alpha version of this algorithm have been described previously (Temko et al., 2011a). The current beta version incorporates a modification to reduce false detections due to persistent artefact (Temko et al., 2013). In summary, the EEG is down-sampled to 32 Hz with an anti-aliasing filter set at 12.8 Hz and is then split into 8 s epochs with 50% overlap between epochs. Fifty-five features are then extracted for each channel from each epoch representing both time and frequency domain characteristics as well as information theory based parameters. Details of main features extracted are given in Table 2. The features extracted from each epoch are then fed into a support vector machine classifier. The output of the SDA is a graph of the probability of seizure calculated using all features in any one 8 s epoch, from zero to 1. This analysis is performed separately for each channel then results are combined for all channels into a single graph (Fig. 1, top panel). A seizure is designated when the probability graph breaches a threshold. The seizure sensitivity threshold is adjustable from 0.1 (most sensitive) to 0.9 (least sensitive). The adjustable threshold allows the algorithm to be tuned on a patient by patient basis. For example, should an EEG recording contain large amounts of artefact causing

Table 2

Main features extracted from the EEG by the SDA.

| Groups | Feature list |
|---------------------|--|
| Frequency domain | <ul style="list-style-type: none"> • Total power (0–12 Hz) • Peak frequency of spectrum • Spectral edge frequency (80%, 90%, 95%) • Power in 2 Hz width sub-bands (0–2 Hz, 1–3 Hz, ...10–12 Hz) • Normalised power in sub-bands • Wavelet energy (the EEG is decomposed into 8 coefficients using the Daubechey 4 wavelet, the energy in the 5th coefficient corresponding to 1–2 Hz is used as a feature) |
| Time domain | <ul style="list-style-type: none"> • Curve length • Number of maxima and minima • Root mean squared amplitude • Hjorth parameters • Zero crossings (raw epoch, Δ, $\Delta\Delta$) • Autoregressive modelling error (model order 1–9) • Skewness • Kurtosis • Nonlinear energy • Variance (Δ, $\Delta\Delta$) |
| Information theory: | <ul style="list-style-type: none"> • Shannon entropy • Singular value decomposition entropy • Fisher information • Spectral entropy |

false detections, the SDA can be desensitised to reduce this number but with a concomitant decrease in the seizure detection rate, as there will always be a negative trade-off between the number of detected seizures and false detections. An SDA annotation was exported for each threshold and was used for comparison with the expert rater's annotation. The SDA and the expert rater's annotations were stored as text files.

2.2. Assessment of the SDA

Assessment of an SDA against a “gold standard” is not a trivial task (Temko et al., 2011b). There is a relative scarcity of seizures in any long duration recording, and in clinical practice recordings will be made in many babies with no seizures at all. The SDA may detect a seizure but the assessed duration might not be in agreement with the “expert” view. The possible output of a comparison is demonstrated in Fig. 2, illustrating the true positive situation (TP) when both the SDA and the expert rater agree there is seizure activity, and true negative (TN) when neither the rater or the SDA classify the EEG as showing seizure. A false positive (FP)

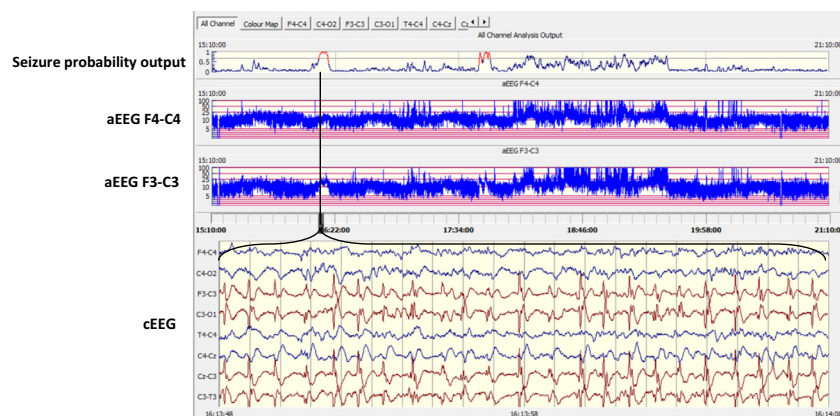


Fig. 1. The SDA incorporated into an EEG viewer. The output of the SDA is a graph of the probability of seizure (upper panel). When a seizure is detected the trace turns red and an annotation is made. The viewer also displays the continuous EEG and aEEG.

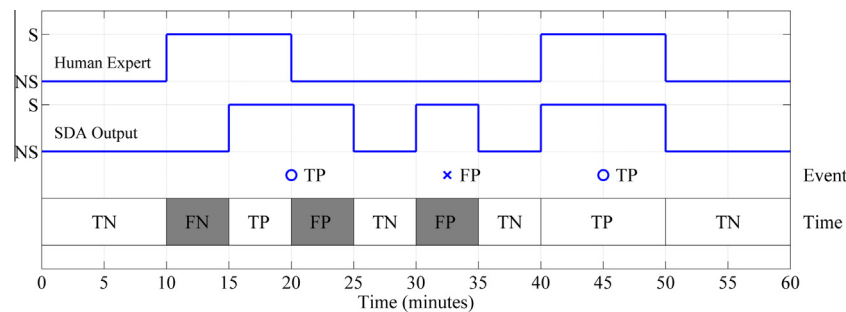


Fig. 2. Temporal and event based assessment of agreement between the annotation of the human expert and the SDA output. S denotes seizure and NS denotes non-seizure. Light/shade in time bar denotes periods of temporal agreement/disagreement: true positives (TP), true negatives (TN), false positives (FP) and false negatives (FN). Markers denote event based agreement: TP and FP. Sensitivity for temporal assessment is 75.0% and specificity for temporal assessment is 75.0%. Sensitivity for event based assessment is 66.7% and a false alarm rate of 1/h.

or type 1 error occurs when the human expert did not annotate a seizure but the SDA output is seizure, and a false negative (FN) or type II error occurs when the expert annotates the recording as seizure but the SDA does not. In order to achieve a rigorous evaluation the probability output time series was set at 60 samples per minute (1 Hz). In order to measure the agreement between the annotations of seizure by the SDA and the expert rater, both records were converted into a binary time series (in this case the time series sampled at 1 Hz). The binary signal was generated by denoting the presence of a seizure at any second with '1' and absence of seizure at any second with '0'.

2.2.1. Conventional measures of agreement

Using the concept of true positive and true negative detection outlined above, conventional measures can be calculated. Sensitivity, defines agreement between the human expert and SDA for identifying the presence of seizure, $TP/(TP + FN)$, and specificity defines agreement between the human expert and SDA for identifying the absence of seizure, $TN/(TN + FP)$. The estimates of sensitivity and specificity can be applied directly to the annotation time series (time based assessment) or in an event based assessment (Fig. 2). The time based metrics correspond to an 'overlap integral' method of assessment (Wilson et al., 2003). The event based metrics correspond to an 'any overlap' method of assessment and must be modified so that specificity is replaced by a measurement of the false detections per hour (false positives per hour) due to a poorly defined 'no seizure' event (Wilson et al., 2003). The sensitivity and specificity can also be used to calculate the area under the receiver operator characteristic (a plot of the specificity vs the sensitivity). The effect of seizure duration on the accuracy of seizure detection (event based analysis) was also examined.

The assessment of agreement was examined on a case-by-case basis. Measures of agreement were then summarised across neonates using the median and interquartile range (the distribution of performance measures will be nonparametric). Agreement was assessed using Cohen's Kappa index.

Performance metrics for the current validation study were also compared against results of the previous 'leave one out' study (Temko et al., 2013).

2.2.2. Application specific measures of SDA usefulness

The agreement between several interpretations of the annotation was compared using the intra-class correlation coefficient (ICC). We quantify interpretation as a summary representation of clinically useful information on seizures over the entire EEG recording of a baby. This includes summary statistics such as seizure burden, seizure number, mean seizure duration, median seizure duration, seizure onset, and seizure period.

The ability of the SDA to support the identification of seizure and non-seizure babies was also examined, ie. detect any seizures in seizure babies and make no false detections in non-seizure babies.

Next the potential of the SDA to support clinical decisions regarding AED administration was examined. With periodic review of the EEG, seizures may not be detected immediately and AEDs are often administered some hours after seizure onset. AEDs may also be administered based on clinical assessment only, potentially erroneously. In order to facilitate this analysis, we examined whether there was seizure activity on the EEG in the 90 min prior to administration of AED (concurrent with AED), or absent in this 90 min period (non-concurrent with AED) to ascertain whether AED was given in a timely or appropriate manner. 90 min was taken as an arbitrary cut off time. This was compared to an examination of the SDA output to confirm whether AEDs had concurrent or non-concurrent SDA seizures. This comparison reflected the ability of an SDA to support clinical decisions regarding AED administration.

3. Results

In total, 107 babies recruited between 5th January 2009 and 30th June 2011 met the inclusion criteria (71 from CUMH and 26 from UCLH). A cohort of 70 babies was then formed by selecting all 35 who had EEG seizures and 35 babies who did not have EEG evidence of seizures. The 35 non-seizure babies were randomly selected from the recordings of the remaining 72 babies in order to match the number of seizure and non-seizure babies in the cohort. The range of demographics for this cohort of neonates is given in Table 3.

The seizure annotations by SM resulted in the detection of 2061 seizures in 35 neonates from a total of 4060 h of multi-channel EEG recordings (Table 4).

3.1. Conventional measures of agreement

Results of the comparison of seizure annotation by SM and GB produced a mean Kappa score of 0.851, which is considered near perfect. The level of agreement (time based analysis) between the annotations of the human expert (SM) and SDA at 9 SDA thresholds, are shown in Table 5A. The maximal level of agreement was at sensitivity threshold 0.4. Further time and event based measures assessed at each SDA threshold are shown in Table 5B.

The results for time based metrics are also shown in Fig. 3. Fig. 3a compares the performance of the unseen validation study to the previous 'leave one out' cross validation (Temko et al., 2013). The median AUC for the validation study, estimated on

Table 3

Demographics and EEG recording information relating to the 70 neonates used in this study.

| | |
|---|--|
| Gestational age (weeks [†] days [†]) | 40 ⁺³ (39 ⁺² to 41 ⁺²) |
| Birthweight (g) [†] | 3526 (3140 to 3920) |
| Gender (male:female) | 37:33 |
| Age at EEG onset (h) [†] | 7.0 (3.6–19.0) |
| EEG recording duration (h) [†] | 51.6 (21.5–84.4) |
| Primary diagnoses | Neonates (N) |
| HIE ^a | 37 |
| | Mild 10 |
| | Moderate 19 |
| | Severe 8 |
| Birth depression ^b | 10 |
| Stroke ^c | 8 |
| Focal lesion ^d | 3 |
| Other ^e | 12 |

[†] Median (interquartile range).

^a 22 treated with therapeutic hypothermia (TH).

^b Birth depression without ensuing encephalopathy.

^c Stroke – arterial ischaemic, haemorrhagic, multiple infarctions.

^d Focal lesion – subdural haemorrhage, intraparenchymal bleed.

^e Other – meningitis/HIE (TH), viral encephalitis, sepsis, benign familial neonatal seizures, benign sleep myoclonus, unknown diagnosis.

Table 4

The summary of seizure characteristics in the 35 babies with EEG confirmation of seizures. Seizure onset (h) refers to post natal age in hours.

| | Median | IQR | min | max |
|-----------------------------|--------|--------------|------|-------|
| Seizure onset (h) | 19.0 | (11.5–35.8) | 6.6 | 153.8 |
| Seizure period (h) | 18.6 | (8.6–35.4) | 0.03 | 120.2 |
| Seizure burden (mins) | 79.8 | (25.3–204.6) | 1.9 | 1404 |
| Seizure number (N) | 22 | (7–75) | 1 | 295 |
| Mean seizure duration (s) | 163 | (95–298) | 28 | 2207 |
| Median seizure duration (s) | 115 | (69–186) | 25 | 2207 |

neonates with seizures (sensitivity can only be estimated on neonates who have seizures) was 0.945 (IQR: 0.921–0.971, min: 0.684 max: 0.999). The mean AUC was 0.933. The performance curves for the two datasets are similar with slightly improved results in the validation set. Fig. 3b shows the specificity for neonates with seizure and neonates without seizure. The curves are similar with slightly higher specificity for non-seizure babies than seizure babies at lower thresholds.

The results for event based metrics on a case by case basis are shown in Fig. 4. Again the performance curves for the validation study compared to the LOO cross validation are similar (Fig. 4a). Fig. 4b shows that false alarm rates are similar for seizure and non-seizure babies.

Fig. 5 shows seizure detection rates and FDs/h for individual babies in the cohort. There is variability in seizure detection and false detection rates across babies. Note the high false detection rates in seizure babies 25 and 26 due to respiration and pulse artefact.

Fig. 6 shows the effect of seizure duration on SDA detection rate. The SDA performance is reduced when detecting short seizures. The most common seizure duration was 1–2 min.

3.1.1. Application specific measures of SDA usefulness

The intra-class correlation between estimates of seizure burden, seizure number, mean/median seizure duration, seizure onset and seizure period are shown in Table 6 for the highest performing threshold of the SDA. The highest performing threshold varies depending on the parameter of interest.

The performance of the SDA to support the identification of seizure babies (any seizure detected) and non-seizure babies (no false detections) at several clinically relevant thresholds is shown in

Table 5

The level of agreement between the annotation of the human expert (SM) and the SDA at 9 thresholds. (A) Cohen's Kappa Index (time based metric), (B) sensitivity and specificity (time based metric), seizure detection rate and false alarms per hour (event based metrics). Data are median (IQR).

| SDA threshold | Kappa ^a | Prevalence index ^a | Bias index ^a | |
|---------------|--------------------------|-------------------------------|-------------------------------------|-------------------------------|
| (A) | | | | |
| 0.1 | 0.098 (0.044–0.204) | 0.688 (0.588–0.780) | 0.222 (0.157–0.364) | |
| 0.2 | 0.309 (0.155–0.509) | 0.893 (0.785–0.949) | 0.050 (0.027–0.098) | |
| 0.3 | 0.524 (0.217–0.677) | 0.936 (0.862–0.977) | 0.011 (0.003–0.031) | |
| 0.4 | 0.630 (0.283–0.739) | 0.956 (0.885–0.984) | 0.006 (0.003–0.019) | |
| 0.5 | 0.579 (0.332–0.724) | 0.954 (0.896–0.983) | 0.007 (0.002–0.019) | |
| 0.6 | 0.552 (0.340–0.700) | 0.952 (0.864–0.981) | 0.006 (0.003–0.019) | |
| 0.7 | 0.405 (0.255–0.621) | 0.959 (0.879–0.983) | 0.001 (0.003–0.027) | |
| 0.8 | 0.280 (0.135–0.406) | 0.957 (0.889–0.989) | 0.011 (0.004–0.038) | |
| 0.9 | 0.060 (0–0.207) | 0.941 (0–0.981) | 0.007 (0–0.042) | |
| SDA threshold | Sensitivity ^a | Specificity ^b | Seizure detection rate ^a | False alarm rate ^b |
| (B) | | | | |
| 0.1 | 96.2 (92.6–98.4) | 77.7 (63.0–86.6) | 97.1 (92.2–100.0) | 4.35 (3.28–5.70) |
| 0.2 | 88.1 (80.5–92.3) | 94.5 (91.1–97.9) | 85.7 (77.1–97.6) | 1.20 (0.63–2.01) |
| 0.3 | 76.1 (68.5–83.9) | 98.5 (97.0–99.5) | 75.0 (59.5–91.7) | 0.36 (0.16–0.74) |
| 0.4 | 68.6 (56.3–80.2) | 99.5 (98.6–99.9) | 64.0 (41.6–85.3) | 0.12 (0.04–0.29) |
| 0.5 | 58.6 (41.2–70.4) | 99.8 (99.3–100.0) | 52.6 (28.3–73.4) | 0.04 (0–0.18) |
| 0.6 | 44.2 (31.4–70.0) | 99.9 (99.7–100.0) | 50.0 (23.1–60.1) | 0 (0–0.06) |
| 0.7 | 32.1 (15.0–51.0) | 100.0 (99.9.0–100.0) | 34.2 (13.4–50.0) | 0 (0–0.01) |
| 0.8 | 16.4 (7.4–30.9) | 100.0 (100.0–100.0) | 23.7 (7.2–38.9) | 0 (0–0) |
| 0.9 | 3.4 (0–12.1) | 100.0 (100.0–100.0) | 5.1 (0–16.2) | 0 (0–0) |

^a Estimated on neonates with seizure (N = 35).

^b Estimated on all neonates (N = 70).

Fig. 7. There is a trade-off between number of seizure and non-seizure babies detected depending on the SDA sensitivity threshold. The best performing SDA sensitivity threshold was at 0.8 (30/35 seizure babies identified, 31/35 non-seizure babies identified). Clinical recognition of seizure/non-seizure babies (identification of a seizure baby was assumed if AED was given, identification of a non-seizure baby was assumed if AED not given) was slightly superior to the SDA (33/35 seizure babies identified, 30/35 non-seizure babies identified). The SDA did not detect any seizures that had been missed by the expert reviewer in the non-seizure baby group.

The potential of the SDA to support clinical decisions regarding AED administration is shown in Fig. 8. A total of 97 AED administrations were recorded (NB. Maintenance doses were not analysed). Of these, 78 were administered during EEG recording. 53/78 were concurrent with EEG seizures (within 90 min preceding AED administration) and 25/78 were administered with no concurrent seizures (in the 90 min preceding AED administration). Again there is a trade-off in the performance of the SDA to support clinical decisions regarding AED administration between supporting concurrent and non-concurrent AED decisions, dependent on SDA sensitivity threshold. The data does suggest however that

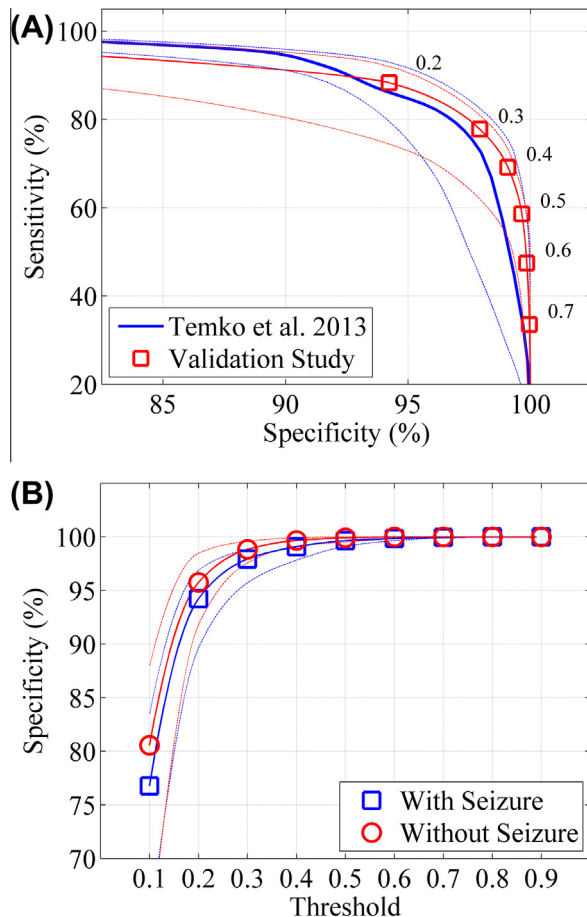


Fig. 3. Time based measures (overlap integral) of SDA performance. The broken lines denote the interquartile range. (A) The median receiver operator curves (the trade-off between sensitivity and specificity) of validation set (estimated on babies with seizure, $N = 35$) compared to 'leave one out' cross validation set (Temko et al., 2013). The numbers on the plot relate to the threshold at which the sensitivity and specificity were estimated. (B) The median specificity of the SDA for validation set with respect to SDA threshold, estimated on babies with seizure ($N = 35$) and babies without seizure ($N = 35$) in validation study.

there is a potential for the SDA to beneficially support these decisions. The SDA, at a threshold of 0.5 and 0.6 performed equally well in terms of its overall effectiveness to correctly identify seizures or seizure free EEG in the 90 min preceding AEDs and therefore to potentially support clinical decisions regarding AED administrations. At a threshold of 0.5, 45/53 (85%) AED administrations concurrent with EEG evidence of seizure would be supported by the SDA and only 6/25 (24%) of AED administrations with non-concurrent seizures would be supported by the SDA, i.e. the SDA has the potential to reduce non-concurrent AED administration by 76% at a cost of not detecting 15% of concurrent seizures.

3.2. Missed seizures and false detections

Examples of seizures that were not detected by the SDA are illustrated in Fig. 9. These were often short or low amplitude or had a dysrhythmic or complex morphology. A quantitative analysis of both missed seizures and false detections will be published separately. Some common causes of false detection are shown in Fig. 10. Respiration and pulse artefacts are recognisable as they are synchronized to the respiration and ECG traces respectively. Sweat artefact produces characteristic large semi-rhythmic waves spanning several seconds. A highly rhythmic background EEG pattern also caused false detections in some cases. This pattern was

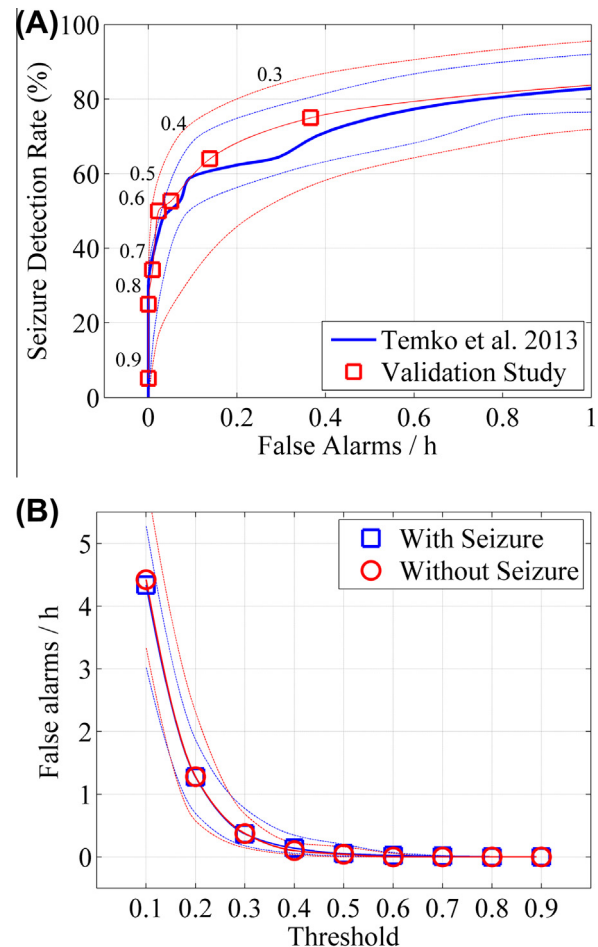


Fig. 4. Event based measures (any overlap) of SDA performance compared to the original LOO cross-validation. The broken lines denote the interquartile range. (A) The trade-off between seizure detection rate and false alarms per hour for babies with seizure ($N = 35$) in validation study compared to 'leave one out' cross validation study (Temko et al., 2013). The numbers on the plot relate to the threshold at which the sensitivity and specificity were estimated. (B) The median false alarm rate with respect to SDA threshold estimated on babies with seizure ($N = 35$) and babies without seizure ($N = 35$) in validation study.

often observed in intermediate sleep, a phase between active and passive sleep, when widespread delta activity is known to increase. In some cases this delta activity had an increased rhythmicity than is commonly observed and consequently caused false detections. This is evident in Fig. 10d where the periodic peaks in the CFM indicating intermediate/quiet sleep correspond to peaks in the SDA probability output and a highly rhythmic EEG pattern is shown in the lower panel.

4. Discussion

In this study a comprehensive set of metrics have been used to measure the performance of our SDA on a large, unedited dataset of prolonged, clinical EEGs from two institutions. To the best of our knowledge this is the largest data set used for SDA validation in babies to date. Only a small subset of previous SDAs has been investigated on a large cohort of babies (Gotman et al., 1997; Lawrence et al., 2009; Mitra et al., 2009; Cherian et al., 2011).

We have used a reduced set of 9 recording electrodes in our study which the algorithm is preset to analyse. While some centres may favour a full set of electrodes (up to 32 recording electrodes)

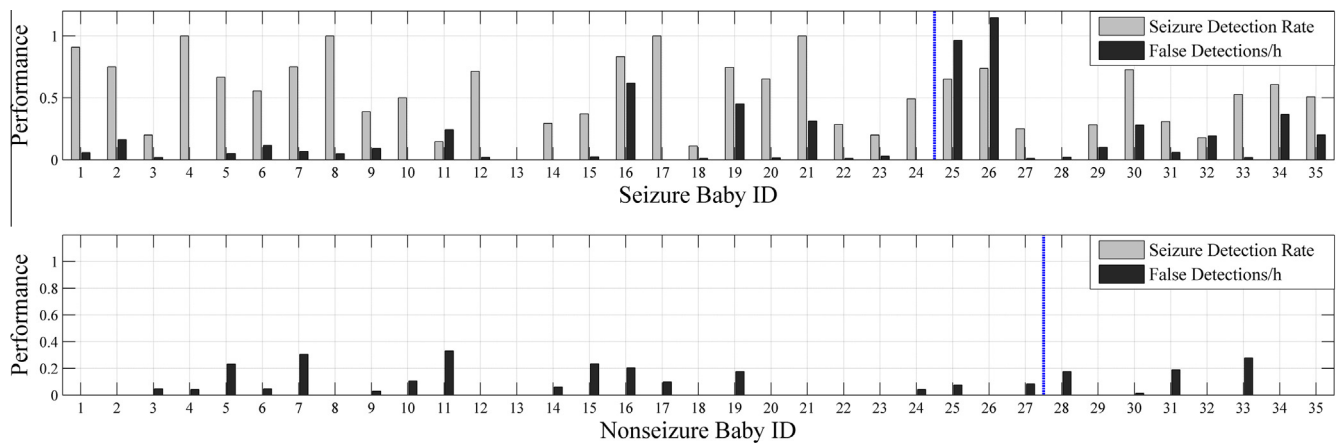


Fig. 5. Seizure detection rates and FDs/h (SDA sensitivity threshold 0.5) for individual babies in the cohort. Note the high false detection rates in seizure babies 25 and 26 due to respiration and pulse artefact. Babies to the left of the vertical line were babies recruited in Cork and those to the right were recruited in London.

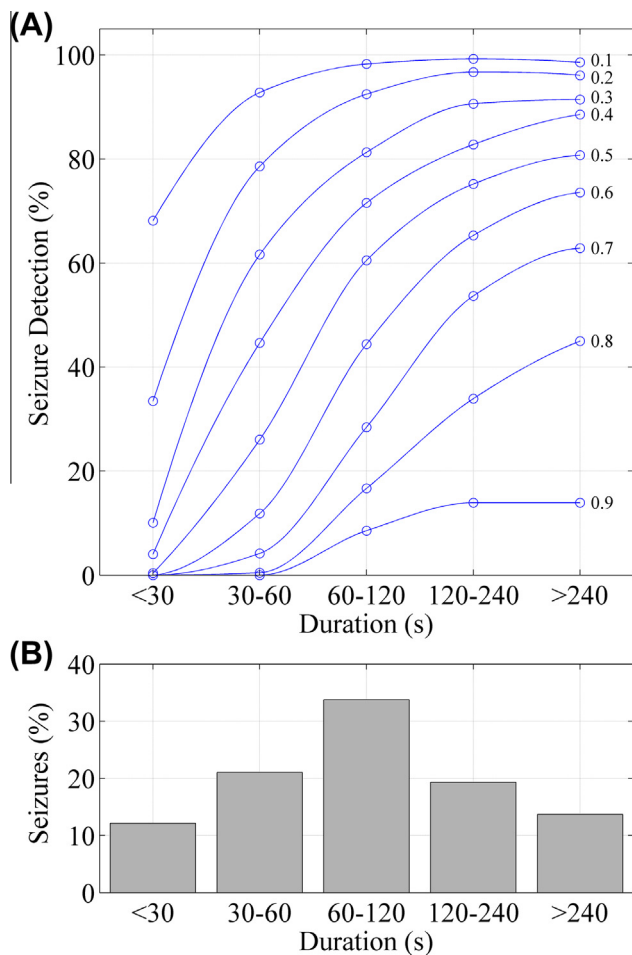


Fig. 6. Analysis of seizure detection rate with respect to seizure duration. (A) SDA performance with respect to seizure duration over nine thresholds. (B) The distribution of seizure durations throughout the concatenated recording.

which are useful for the purposes of seizure onset localisation, our primary goal is seizure detection. A study by Tekgul et al. (2005) comparing seizure detection between a full 10:20 montage and a reduced 9 electrode set, found very few seizures were missed with a sensitivity of 96.8% for the reduced montage compared to the full set. The benefits of using more electrodes must be weighed against

Table 6

Agreement between interpretation of annotations of the human expert (SM) and the SDA. Interpretation relates to summary statistics of the temporal evolution of seizures in each EEG recording. The average intra-class correlation coefficient (ICC) is presented and is estimated on the entire cohort of babies ($N = 70$). The summary statistics of seizures are estimated from the SDA annotation but only on babies who have seizure as detected by the human expert. The threshold that results in the highest ICC for each measure is shown. The SDA detects at least one seizure in 64, 54, 43, 38 babies at thresholds of 0.4, 0.5, 0.6, 0.7 respectively.

| | Median | IQR | ICC | SDA threshold |
|-----------------------------|--------|--------------|-------|---------------|
| Seizure onset (h) | 15.1 | (7.7–32.5) | 0.722 | 0.4 |
| Seizure period (h) | 26.3 | (7.3–49.8) | 0.802 | 0.6 |
| Seizure burden (mins) | 81.1 | (26.6–181.3) | 0.859 | 0.5 |
| Seizure number (N) | 35 | (7–72) | 0.930 | 0.4 |
| Mean seizure duration (s) | 169 | (121–243) | 0.511 | 0.5 |
| Median seizure duration (s) | 124 | (92–146) | 0.323 | 0.7 |

the time and technical constraints to the NNU staff of applying more electrodes out of hours.

We have found the performance of our algorithm to compare favourably with those previously reported by others (Table 1), although in previous papers not all metrics were reported for full comparison. For example Gotman (Gotman et al., 1997) reported a SDR of 66% with a FD rate of 2.3 FD/h. At a threshold of 0.3, the SDA reported here achieved a higher SDR of 75% at a much lower FD rate of 0.4 FD/h.

Navakatikyan (Navakatikyan et al., 2006), reported a SDR of 90% at 2 FD/h. In comparison, at a threshold of 0.2 our system achieved a slightly lower SDR rate of 85%, but with a much lower false detection rate of 1.2 FD/h. In a clinical validation study of the Navakatikyan algorithm, Lawrence (Lawrence et al., 2009) compared the output of the algorithm with 12 h recordings of conventional video-EEG, and found a seizure detection rate of 55% with a false detection rate of 0.1 FD/h. This was quite different to the initial performance results (Navakatikyan et al., 2006). At a threshold of 0.5, the system reported here achieved a slightly lower SDR of 53% but again with a lower FD rate of 0.04/h.

Deburchgraeve et al. (2008) initially reported an SDR of 85% with a FD rate of 0.7 FD/h. At a threshold of 0.2, our system achieved a similar SDR of 86% with a higher FD rate of 1.2 FD/h. However in a clinical validation of this algorithm, Cherian et al. (2011) reported a lower SDR rate of 66% with a FD rate of 0.58 FD/h. At threshold 0.4 our system achieved only a slightly lower SDR rate of 64.0% but with a considerably lower FD rate of 0.12 FD/h.

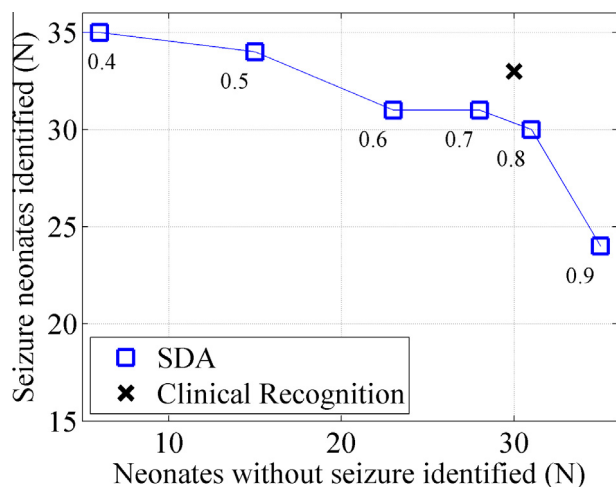


Fig. 7. The accuracy of the SDA for the identification of seizure and non-seizure babies at several thresholds. There were 35 neonates with EEG evidence of seizure and 35 neonates without EEG evidence of seizure. Clinical recognition is based on AED administration and is superior to the SDA.

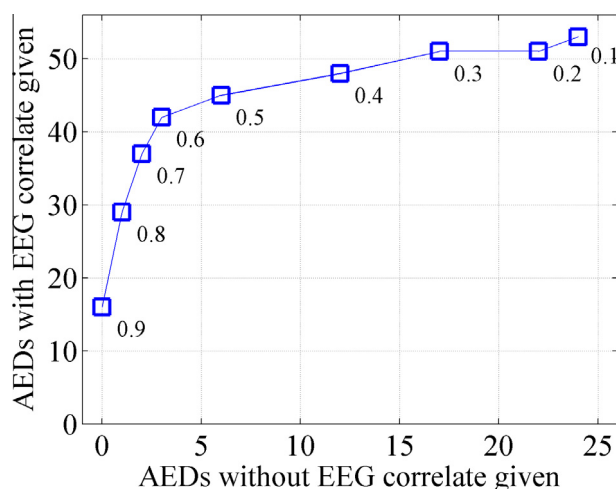


Fig. 8. Potential of the SDA to support decisions on AED administration. A total of 97 AED administrations were recorded. Of these, 78 were administered concurrently with EEG recording and 53 were concurrent with EEG seizures (seizures occurring in the 90 min prior to AED administration). At a threshold of 0.5, 45 (85%) AED administrations concurrent with EEG evidence of seizure and only 6 (24%) of AED administrations with no EEG evidence of seizure would be supported by the SDA.

The SDA presented by Mitra et al. (2009) gave a SDR of 80% with a FD rate of 0.78 FD/h. The SDR is midway between the SDR rates reported here at threshold 0.3 (75.0%) and 0.2 (85%) with FD rates at 0.36 FD/h and 1.20 FD/h respectively, thus at 80% detection rate our FD rate would be comparable to that of Mitra.

In this study, the performance of our SDA on an unseen dataset mimicked the performance seen in the previous 'leave one out' cross validation (Temko et al., 2013). In 'leave one out' cross validation the data for each patient is tested using the data for all other patients as training data for the algorithm and the process repeated for each patient and the results averaged. These results suggest firstly that the data set used to train the algorithm contained a representative population of seizure and background EEG patterns and secondly that the SDA performs equally well on unseen data, as will be encountered in clinical use.

This study, in conjunction with the paper by Temko et al. (2013), are also the first papers to evaluate the SDA in a so-called 'mismatch' situation, where the seizure annotations of one expert are used to train the algorithm and the annotations of another expert are used to test the SDA. In addition, in this paper, we have, for the first time, tested the algorithm on data collected from two different centres, CUMH and UCLH, with potential differences in EEG application and recording. Given these two factors, the similarity of current SDA performance with previous performance demonstrates a practically acceptable degree of robustness of the algorithm.

The current performance, analysed with a very rigorous definition of true positive and true negative detections, was very good for most babies with seizures (Fig. 5). Two seizure babies (25 and 26) had high false detection rates due to respiration and pulsatile artefact. In future, it may be possible to teach clinical staff simple artefact "pattern" recognition (Fig. 10) so that false detection would not lead to overtreatment. For example, respiration and pulsatile artefact are both easily recognised as they are synchronized to the respiration and ECG traces respectively and are invariant as they do not show the evolving features of many seizures. Similarly sweat artefact produces characteristic high amplitude, semi-rhythmic slow waves spanning several seconds, a far slower frequency than typical seizures. Indeed, the results of the pilot study by Lawrence et al. in which pre-training was given, support this with only 1 single dose of AED given inappropriately in 232 false detection events (Lawrence et al., 2009).

The analysis of AED administration has shown that on 25 occasions AEDs were given without EEG seizures in the preceding 90 min and that the SDA has the potential to support clinical decisions to reduce AED administrations with 'non-concurrent' seizures. We are not suggesting that in 25 cases AEDs were given 'inaccurately' by clinical staff. In only one case did we identify that an AED had been given on clinical suspicion of seizure alone (without any EEG correlate at all). In most cases we suspect that there was simply a delay in detection of seizure and AED administration due to the nature of periodic EEG review which could potentially have been reduced with the support of the algorithm alerting clinical staff earlier.

The performance of the SDA has been presented over a range of sensitivity thresholds and the metrics used allow 'best performing' thresholds to be determined. However the choice of sensitivity threshold used in a clinical environment is critically dependent on the fact that best performing thresholds differ with tasks and threshold choice is therefore dependent on the requirement of the user. For example, the best performing threshold for detecting the maximal number of seizure/non-seizure babies correctly (Fig. 7) was threshold 0.8 while for supporting decisions regarding AED administration (Fig. 8), thresholds of 0.5/0.6 were optimal. The intra-class correlations in Table 6 show a variety of best performing thresholds for different parameters of interest. Notably, for detecting seizure onset and seizure number, a threshold of 0.4 performed best. For the task of correctly detecting the greatest 'amount' of seizure/non-seizure activity (seizure burden), time based analysis provides the most accurate measure and the Kappa score comparing human and SDA annotations indicated that the best performing threshold was also 0.4 (Kappa 0.630).

In clinical practice however, it is not likely that clinicians will be concerned with accurately detecting every single second of each seizure and are likely to care most that the SDA makes 'some' detection during a seizure and that overall the output of the SDA most accurately represents the numbers of seizures occurring with an acceptable false detection rate. This would allow treatment to be titrated to the presence of ongoing seizures, and in this respect the event based metrics may be of more interest clinically. The concept of what is deemed *acceptable* in terms of the rate of false

alarms is also dependent on user preference and may vary between users, affecting the choice of sensitivity threshold.

We consider the output from the SDA at thresholds from 0.5 to 0.3 to be within a clinically acceptable range, giving detection rates between 52.6% and 75.0% with false detections, on average, approximately every 20 and 3 h respectively (Table 5B).

This range is proposed on the basis of a perceived expectation that a minimum of 50% seizure detection is required and that a false detection rate of greater than 0.5/h might be considered excessive.

The data presented here represents only one stage in the assessment of the SDAs performance which will be further tested in a

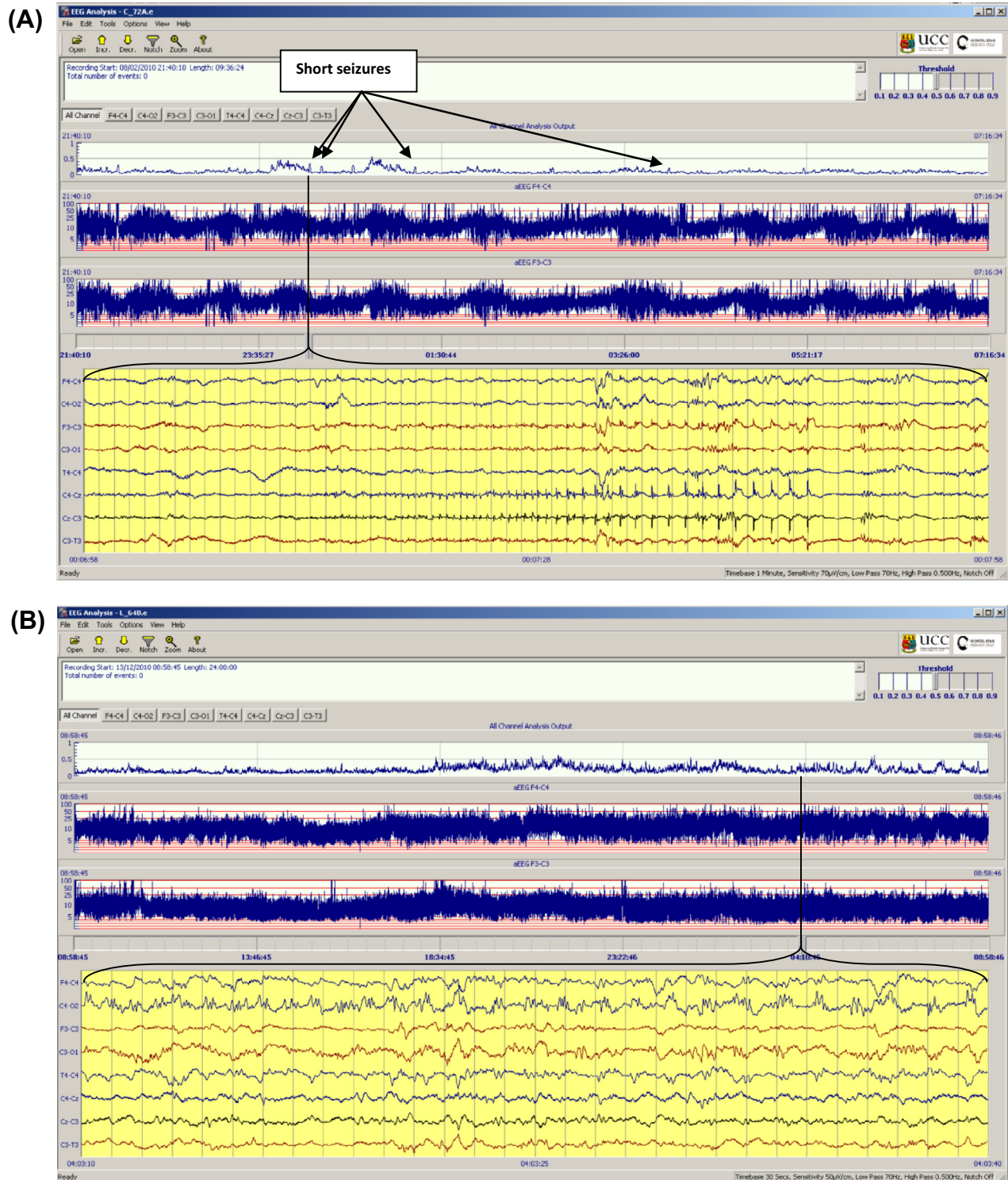


Fig. 9. Seizures missed by the SDA. (A) Brief 30 s seizure. 0 of 4 seizures were detected in this record (thr 0.5), though the algorithm output would cause the clinician to interrogate the EEG at various points despite the fact that the fixed threshold was not reached. (B) Subtle, dysrhythmic 2 min seizure with complex morphology, 0 of 1 seizures were detected in this record (thr 0.5). (C) Low amplitude seizure, 31 of 55 seizures were detected in this record (note. Non detected seizures produce clear peaks on the probability trace for interrogation).

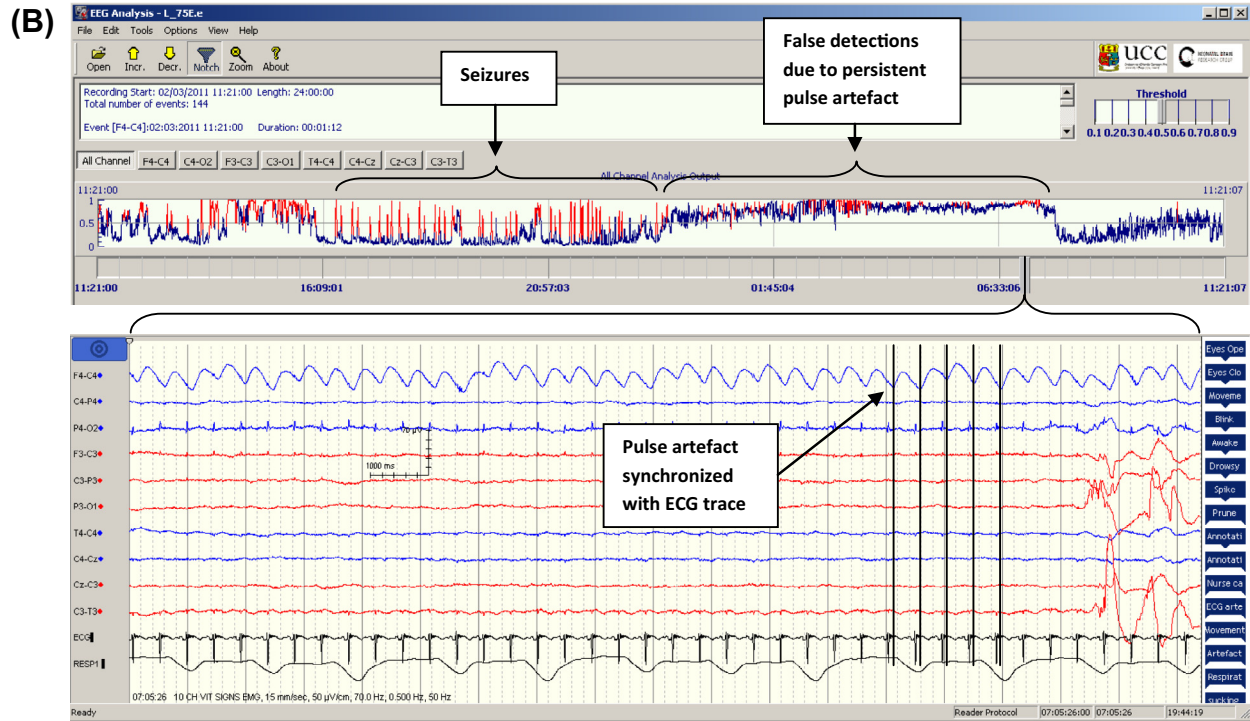


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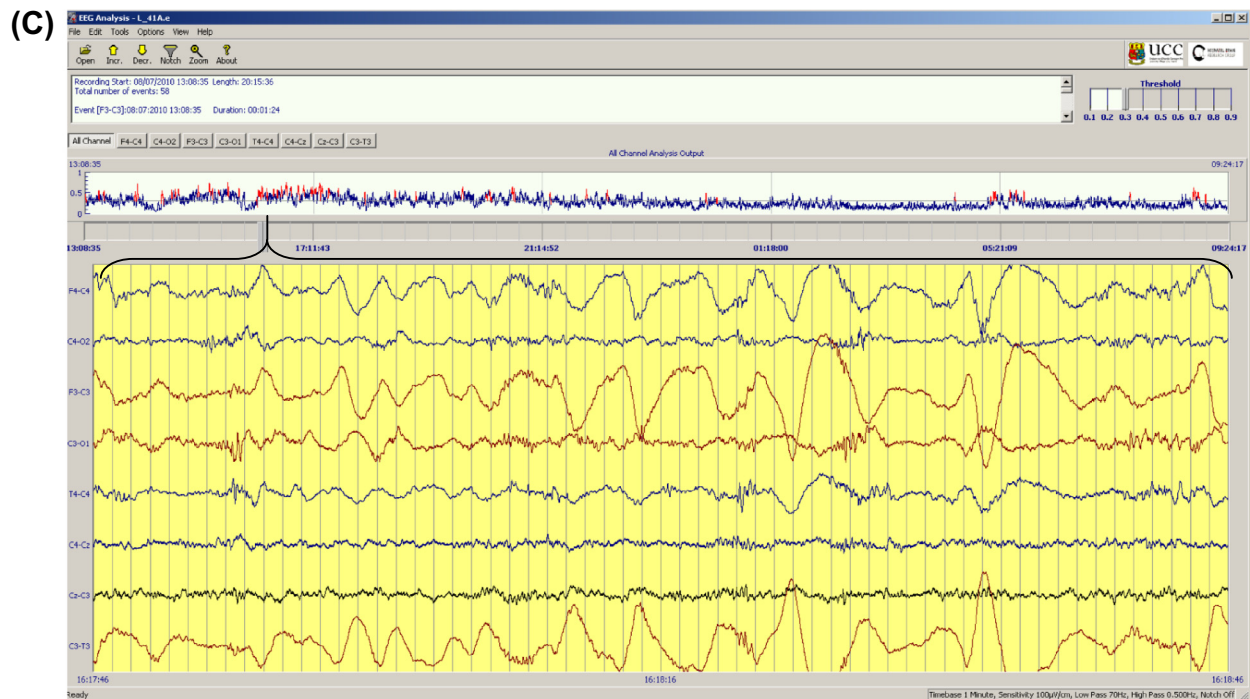


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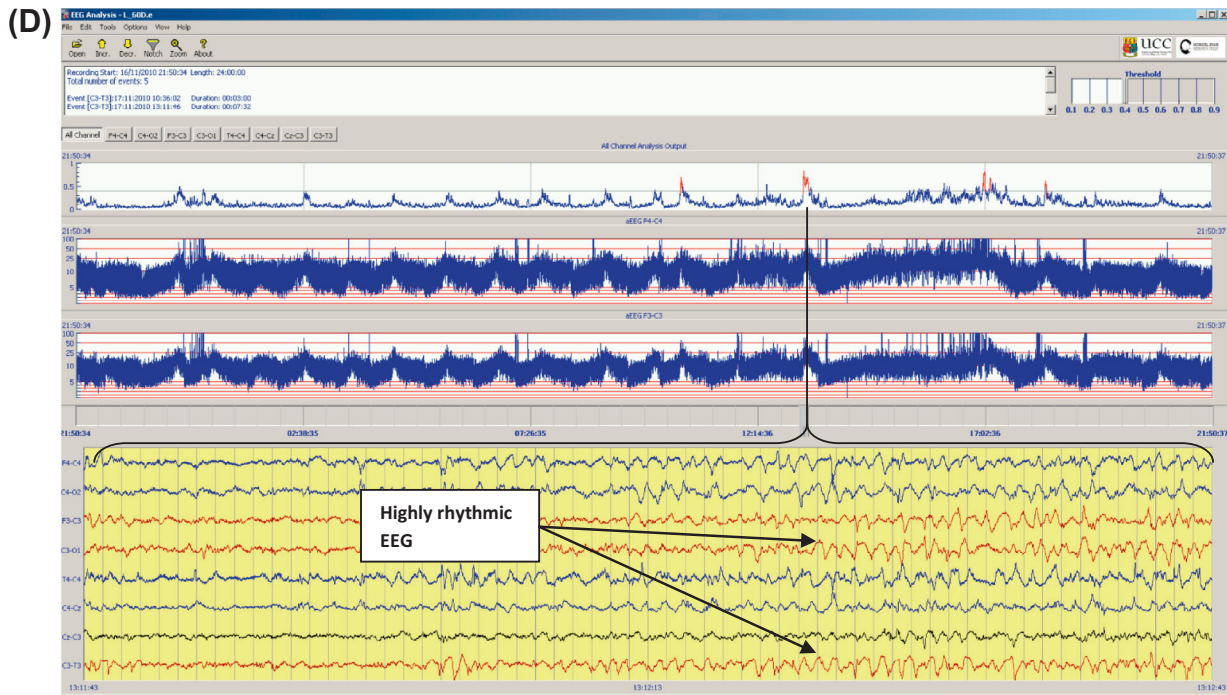


Fig. 10 (continued)

probability of seizure. A clinician reviewing the output of the SDA at the cotside is likely to interrogate both prominent peaks that breach the threshold on the graph and others that do not (eg. Fig. 9c). With a “pattern recognition” support package, the ability of clinicians to differentiate seizures from artefacts can, potentially, be improved. For these reasons, the role of the reviewer is central to the interpretation of the output of the SDA and consequently how many seizures, false detections, seizure babies and non-seizure babies are identified correctly. Our intention is that the seizure detection performance of clinicians with the assistance of our algorithm will be superior to the algorithm’s simple binary ‘alerts’ based on fixed thresholds presented here.

5. Conclusion

We have validated a neonatal SDA on a large EEG dataset and have shown that it achieves a clinically useful level of seizure detection with acceptable false detection rates. Future multi-centre evaluation of the SDA in a ‘live’ clinical environment will critically investigate the clinician’s interpretation of the full SDA output to determine the usefulness of the SDA in the NICU.

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References

- Cherian PJ, Deburchgraeve W, Swarte RM, De Vos M, Govaert P, Van Huffel S, et al. Validation of a new automated neonatal seizure detection system: a clinician’s perspective. *Clin Neurophysiol* 2011;122:1490–9.
- Clancy RR, Legido A. The exact ictal and interictal duration of electroencephalographic neonatal seizures. *Epilepsia* 1987;28:537–41.
- Deburchgraeve W, Cherian PJ, De Vos M, Swarte RM, Blok JH, Visser GH, et al. Automated neonatal seizure detection mimicking a human observer reading EEG. *Clin Neurophysiol* 2008;119:2447–54.
- Glass HC, Bonifacio SL, Shimotake T, Ferriero DM. Neurocritical care for neonates. *Curr Treat Options Neurol* 2011;13:574–89.
- Glass HC, Glidden D, Jeremy RJ, Barkovich AJ, Ferriero DM, Miller SP. Clinical Neonatal Seizures are Independently Associated with Outcome in Infants at Risk for Hypoxic-Ischemic Brain Injury. *J Pediatr* 2009;155:318–23.
- Gotman J, Flanagan D, Rosenblatt B, Bye A, Mizrahi EM. Evaluation of an automatic seizure detection method for the newborn EEG. *Electroencephalogr Clin Neurophysiol* 1997;103:363–9.
- Lawrence R, Mathur A, Nguyen The Tich S, Zempel J, Inder T. A pilot study of continuous limited-channel aEEG in term infants with encephalopathy. *J Pediatr* 2009;154(835–41):e1.
- Liu A, Hahn JS, Heldt GP, Coen RW. Detection of neonatal seizures through computerized EEG analysis. *Electroencephalogr Clin Neurophysiol* 1992;82:30–7.
- Mitra J, Glover JR, Ktonas PY, Thitai Kumar A, Mukherjee A, Karayiannis NB, et al. A multistage system for the automated detection of epileptic seizures in neonatal electroencephalography. *J Clin Neurophysiol* 2009;26:218–26.
- Murray DM, Boylan GB, Ali I, Ryan CA, Murphy BP, Connolly S. Defining the gap between electrographic seizure burden, clinical expression and staff recognition of neonatal seizures. *Arch Dis Child Fetal Neonatal Ed* 2008;93:F187–91.
- Navakatikyan MA, Colditz PB, Burke CJ, Inder TE, Richmond J, Williams CE. Seizure detection algorithm for neonates based on wave-sequence analysis. *Clin Neurophysiol* 2006;117:1190–203.
- Rennie JM, Chorley G, Boylan GB, Pressler R, Nguyen Y, Hooper R. Non-expert use of the cerebral function monitor for neonatal seizure detection. *Arch Dis Child Fetal Neonatal Ed* 2004;89:F37–40.
- Shah DK, Wusthoff CJ, Clarke P, Wyatt SM, Dias RJ, et al. Electrographic seizures are associated with brain injury in newborns undergoing therapeutic hypothermia. *Arch Dis Child Fetal Neonatal Ed* 2014;99:F219–24.
- Smit LS, Vermeulen RJ, Fetter WP, Strijers RL, Stam CJ. Neonatal seizure monitoring using non-linear EEG analysis. *Neuropediatrics* 2004;35:329–35.
- Tekgul H, Bourgeois BF, Gauvreau K, Bergin AM. Electroencephalography in neonatal seizures: comparison of a reduced and a full 10/20 montage. *Pediatr Neurol* 2005;32:155–61.
- Temko A, Boylan G, Marnane W, Lightbody G. Robust neonatal EEG seizure detection through adaptive background modeling. *Int J Neural Syst* 2013;23:1350018.

- Temko A, Thomas E, Marnane W, Lightbody G, Boylan G. EEG-based neonatal seizure detection with Support Vector Machines. *Clin Neurophysiol* 2011a;122:464–73.
- Temko A, Thomas E, Marnane W, Lightbody G, Boylan GB. Performance assessment for EEG-based neonatal seizure detectors. *Clin Neurophysiol* 2011b;122:474–82.
- Wilson SB, Scheuer ML, Plummer C, Young B, Pacia S. Seizure detection: correlation of human experts. *Clin Neurophysiol* 2003;114:2156–64.
- Wirrell EC, Armstrong EA, Osman LD, Yager JY. Prolonged seizures exacerbate perinatal hypoxic-ischemic brain damage. *Pediatr Res* 2001;50:445–54.

Section 6

References

- Abend NS, Arndt DH, Carpenter JL, Chapman KE, Cornett KM, Gallentine WB, Giza CC, Goldstein JL, Hahn CD, Lerner JT, Loddenkemper T, Matsumoto JH, McBain K, Nash KB, Payne E, Sanchez SM, Fernandez IS, Shults J, Williams K, Yang A, Dlugos DJ. (2013a) Electrographic seizures in pediatric ICU patients: cohort study of risk factors and mortality. *Neurology* 81:383-391.
- Abend NS, Gutierrez-Colina AM, Monk HM, Dlugos DJ, Clancy RR. (2011) Levetiracetam for treatment of neonatal seizures. *J Child Neurol* 26:465-470.
- Abend NS, Wusthoff CJ. (2012) Neonatal seizures and status epilepticus. *J Clin Neurophysiol* 29:441-448.
- Abend NS, Wusthoff CJ, Goldberg EM, Dlugos DJ. (2013b) Electrographic seizures and status epilepticus in critically ill children and neonates with encephalopathy. *Lancet Neurol* 12:1170-1179.
- Abraham J, Fox PD, Condello C, Bartolini A, Koh S. (2012) Minocycline attenuates microglia activation and blocks the long-term epileptogenic effects of early-life seizures. *Neurobiol Dis* 46:425-430.
- ACOG. (2006) ACOG Committee Opinion No. 348, November: Umbilical cord blood gas and acid-base analysis. *Obstet Gynecol* 108:1319-1322.
- Agarwal M, Fox SM. (2013) Pediatric seizures. *Emerg Med Clin North Am* 31:733-754.
- Alfonso I, Papazian O, Litt R, Meyer K, Paez J. (2000) Single photon emission computed tomographic evaluation of brainstem release phenomenon and seizure in neonates. *J Child Neurol* 15:56-58.
- Allegaert K, Verbesselt R, Naulaers G, van den Anker JN, Rayyan M, Debeer A, de HJ. (2008) Developmental pharmacology: neonates are not just small adults.. *Acta Clin Belg* 63:16-24.
- Aly H, Mohsen L, Badrawi N, Gabr H, Ali Z, Akmal D. (2012) Viability and neural differentiation of mesenchymal stem cells derived from the umbilical cord following perinatal asphyxia. *J Perinatol* 32:671-676.
- Ancora G, Soffritti S, Lodi R, Tonon C, Grandi S, Locatelli C, Nardi L, Bisacchi N, Testa C, Tani G, Ambrosetto P, Faldella G. (2010) A combined a-EEG and MR spectroscopy study in term newborns with hypoxic-ischemic encephalopathy. *Brain Dev* 32:835-842.
- Antonucci R, Porcella A, and Pilloni MD. Perinatal asphyxia in the term newborn. *Journal of Pediatric and Neonatal Individualized Medicine* 2014 3[2], e030269 doi: 10.7363/030269. 2014.
Ref Type: Journal (Full)
- Apgar V. A proposal for a new method of evaluation of the newborn infant. *Curr.Res.Anesth.Analg.* 32[4], 260-267. 1953.
Ref Type: Journal (Full)
- Aso K, Scher MS, Barmada MA. (1990) Cerebral infarcts and seizures in the neonate. *J Child Neurol* 5:224-228.
- Aujla PK, Fetell MR, Jensen FE. (2009) Talampanel suppresses the acute and chronic effects of seizures in a rodent neonatal seizure model. *Epilepsia* 50:694-701.

- Azzopardi D, Brocklehurst P, Currie A, and Edwards D. UK TOBY Cooling Register Protocol Version 3 [online]. <https://www.npeu.ox.ac.uk/tobyregister> . 2007.
- Azzopardi D. (2015) Clinical applications of cerebral function monitoring in neonates. *Semin Fetal Neonatal Med*.
- Azzopardi D, Robertson NJ, Kapetanakis A, Griffiths J, Rennie JM, Mathieson SR, Edwards AD. (2013) Anticonvulsant effect of xenon on neonatal asphyxial seizures. *Arch Dis Child Fetal Neonatal Ed* 98:F437-F439.
- Azzopardi D, Strohm B, Edwards AD, Halliday H, Juszczak E, Levene M, Thoresen M, Whitelaw A, Brocklehurst P. (2009) Treatment of asphyxiated newborns with moderate hypothermia in routine clinical practice: how cooling is managed in the UK outside a clinical trial. *Arch Dis Child Fetal Neonatal Ed* 94:F260-F264.
- Azzopardi D, Strohm B, Marlow N, Brocklehurst P, Deierl A, Eddama O, Goodwin J, Halliday HL, Juszczak E, Kapellou O, Levene M, Linsell L, Omar O, Thoresen M, Tusor N, Whitelaw A, Edwards AD. (2014) Effects of hypothermia for perinatal asphyxia on childhood outcomes. *N Engl J Med* 371:140-149.
- Back SA, Craig A, Kayton RJ, Luo NL, Meshul CK, Allcock N, Fern R. (2007) Hypoxia-ischemia preferentially triggers glutamate depletion from oligodendroglia and axons in perinatal cerebral white matter. *J Cereb Blood Flow Metab* 27:334-347.
- Barks JD, Liu YQ, Shangguan Y, Silverstein FS. (2010) Phenobarbital augments hypothermic neuroprotection. *Pediatr Res* 67:532-537.
- Barlow JS. (1997) The early history of EEG data-processing at the Massachusetts Institute of Technology and the Massachusetts General Hospital. *Int J Psychophysiol* 26:443-454.
- Barmada MA, Moossy J, Shuman RM. (1979) Cerebral infarcts with arterial occlusion in neonates. *Ann Neurol* 6:495-502.
- Barnette AR, Inder TE. (2009) Evaluation and management of stroke in the neonate. *Clin Perinatol* 36:125-136.
- Bartha AI, Shen J, Katz KH, Mischel RE, Yap KR, Ivacko JA, Andrews EM, Ferriero DM, Ment LR, Silverstein FS. (2007) Neonatal seizures: multicenter variability in current treatment practices. *Pediatr Neurol* 37:85-90.
- Battin M, Bennet L, Gunn AJ. (2004) Rebound seizures during rewarming. *Pediatrics* 114:1369.
- Beaulieu MJ. (2013) Levetiracetam. *Neonatal Netw* 32:285-288.
- Ben-Ari Y. (2006) Seizures beget seizures: the quest for GABA as a key player. *Crit Rev Neurobiol* 18:135-144.
- Ben-Ari Y. (2012) Blocking seizures with the diuretic bumetanide: promises and pitfalls. *Epilepsia* 53:394-396.
- Ben-Ari Y, Crepel V, Represa A. (2008) Seizures beget seizures in temporal lobe epilepsies: the boomerang effects of newly formed aberrant kainatergic synapses. *Epilepsy Curr* 8:68-72.
- Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R. (2007) GABA: a pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol Rev* 87:1215-1284.
- Ben-Ari Y, Holmes GL. (2006) Effects of seizures on developmental processes in the immature brain. *Lancet Neurol* 5:1055-1063.
- Bender RA, Brewster A, Santoro B, Ludwig A, Hofmann F, Biel M, Baram TZ. (2001) Differential and age-dependent expression of hyperpolarization-activated, cyclic nucleotide-gated cation channel isoforms 1-4 suggests evolving roles in the developing rat hippocampus. *Neuroscience* 106:689-698.
- Bennet L, Oliver MH, Gunn AJ, Hennies M, Breier BH. (2001) Differential changes in insulin-like growth factors and their binding proteins following asphyxia in the preterm fetal sheep. *J Physiol* 531:835-841.
- Bennet L, Roelfsema V, George S, Dean JM, Emerald BS, Gunn AJ. (2007) The effect of cerebral hypothermia on white and grey matter injury induced by severe hypoxia in preterm fetal sheep. *J Physiol* 578:491-506.
- Bennet L, Roelfsema V, Pathipati P, Quaedackers JS, Gunn AJ. (2006) Relationship between evolving epileptiform activity and delayed loss of mitochondrial activity after asphyxia measured by near-infrared spectroscopy in preterm fetal sheep. *J Physiol* 572:141-154.
- Bernard SA, Gray TW, Buist MD, Jones BM, Silvester W, Gutteridge G, Smith K. (2002) Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. *N Engl J Med* 346:557-563.
- Biagioni E, Ferrari F, Boldrini A, Roversi MF, Cioni G. (1998) Electroclinical correlation in neonatal seizures. *Eur J Paediatr Neurol* 2:117-125.
- Billard C, Dulac O, Diebler C. (1982) [Ischemic cerebral softening in newborn infants. Possible etiology of neonatal convulsive states]. *Arch Fr Pediatr* 39:677-683.

- Billiards SS, Haynes RL, Folkerth RD, Trachtenberg FL, Liu LG, Volpe JJ, Kinney HC. (2006) Development of microglia in the cerebral white matter of the human fetus and infant. *J Comp Neurol* 497:199-208.
- Bittigau P, Sifringer M, Genz K, Reith E, Pospischil D, Govindarajalu S, Dzietko M, Pesditschek S, Mai I, Dikranian K, Olney JW, Ikonomidou C. (2002) Antiepileptic drugs and apoptotic neurodegeneration in the developing brain. *Proc Natl Acad Sci U S A* 99:15089-15094.
- Bittigau P, Sifringer M, Ikonomidou C. (2003) Antiepileptic drugs and apoptosis in the developing brain. *Ann N Y Acad Sci* 993:103-114.
- Bjorkman ST, Miller SM, Rose SE, Burke C, Colditz PB. (2010) Seizures are associated with brain injury severity in a neonatal model of hypoxia-ischemia. *Neuroscience* 166:157-167.
- Booth D, Evans DJ. (2004) Anticonvulsants for neonates with seizures. *Cochrane Database Syst Rev* CD004218.
- Boylan G, Burgoyne L, Moore C, O'Flaherty B, Rennie J. (2010) An international survey of EEG use in the neonatal intensive care unit. *Acta Paediatr* 99:1150-1155.
- Boylan GB, Kharoshankaya L, Wusthoff CJ. (2015) Seizures and hypothermia: Importance of electroencephalographic monitoring and considerations for treatment. *Semin Fetal Neonatal Med* 20:103-108.
- Boylan GB, Pressler RM. (2013) Neonatal seizures: the journey so far. *Semin Fetal Neonatal Med* 18:173-174.
- Boylan GB, Pressler RM, Rennie JM, Morton M, Leow PL, Hughes R, Binnie CD. (1999) Outcome of electroclinical, electrographic, and clinical seizures in the newborn infant. *Dev Med Child Neurol* 41:819-825.
- Boylan GB, Rennie JM. (2006) Automated neonatal seizure detection. *Clin Neurophysiol* 117:1412-1413.
- Boylan GB, Rennie JM, Chorley G, Pressler RM, Fox GF, Farrer K, Morton M, Binnie CD. (2004) Second-line anticonvulsant treatment of neonatal seizures: a video-EEG monitoring study. *Neurology* 62:486-488.
- Boylan GB, Rennie JM, Pressler RM, Wilson G, Morton M, Binnie CD. (2002) Phenobarbitone, neonatal seizures, and video-EEG. *Arch Dis Child Fetal Neonatal Ed* 86:F165-F170.
- Boylan GB, Stevenson NJ, Vanhatalo S. (2013) Monitoring neonatal seizures. *Semin Fetal Neonatal Med* 18:202-208.
- Brooks-Kayal AR, Shumate MD, Jin H, Rikhter TY, Kelly ME, Coulter DA. (2001) Gamma-Aminobutyric acid(A) receptor subunit expression predicts functional changes in hippocampal dentate granule cells during postnatal development. *J Neurochem* 77:1266-1278.
- Busto R, Globus MY, Dietrich WD, Martinez E, Valdes I, Ginsberg MD. (1989) Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain. *Stroke* 20:904-910.
- Bye A, Flanagan D. (1995a) Electroencephalograms, clinical observations and the monitoring of neonatal seizures. *J Paediatr Child Health* 31:503-507.
- Bye AM, Flanagan D. (1995b) Spatial and temporal characteristics of neonatal seizures. *Epilepsia* 36:1009-1016.
- Castro C, Jr., Gonzalez-Hernandez T, Gonzalez BD, Gonzalez CC. (2012) Neonatal apneic seizure of occipital lobe origin: continuous video-EEG recording. *Pediatrics* 129:e1616-e1620.
- Castro C, Jr., Hernandez Borges AA, Domenech ME, Gonzalez CC, Perera SR. (2005) Midazolam in neonatal seizures with no response to phenobarbital. *Neurology* 64:876-879.
- Cha BH, Silveira DC, Liu X, Hu Y, Holmes GL. (2002) Effect of topiramate following recurrent and prolonged seizures during early development. *Epilepsy Res* 51:217-232.
- Charriaut-Marlangue C, Nguyen T, Bonnin P, Duy AP, Leger PL, Csaba Z, Pansiot J, Bourgeois T, Renolleau S, Baud O. (2014) Sildenafil mediates blood-flow redistribution and neuroprotection after neonatal hypoxia-ischemia. *Stroke* 45:850-856.
- Chaudhari T, McGuire W. (2008) Allopurinol for preventing mortality and morbidity in newborn infants with suspected hypoxic-ischaemic encephalopathy. *Cochrane Database Syst Rev* CD006817.
- Cherian PJ, Deburchgraeve W, Swarte RM, De VM, Govaert P, Van HS, Visser GH. (2011) Validation of a new automated neonatal seizure detection system: a clinician's perspective. *Clin Neurophysiol* 122:1490-1499.
- Cherubini E, Ben-Ari Y. (2011) The immature brain needs GABA to be excited and hyper-excited. *J Physiol* 589:2655-2656.
- Chu CP, Jin WZ, Bing YH, Jin QH, Kannan H, Qiu DL. (2013) Effects of stresscopin on rat hypothalamic paraventricular nucleus neurons in vitro. *PLoS One* 8:e53863.

- Cilio MR, Ferriero DM. (2010) Synergistic neuroprotective therapies with hypothermia. *Semin Fetal Neonatal Med* 15:293-298.
- Clancy R, Malin S, Laraque D, Baumgart S, Younkin D. (1985) Focal motor seizures heralding stroke in full-term neonates. *Am J Dis Child* 139:601-606.
- Clancy RR. (1996) The contribution of EEG to the understanding of neonatal seizures. *Epilepsia* 37 Suppl 1:S52-S59.
- Clancy RR. (2006a) Prolonged electroencephalogram monitoring for seizures and their treatment. *Clin Perinatol* 33:649-665.
- Clancy RR. (2006b) Summary proceedings from the neurology group on neonatal seizures. *Pediatrics* 117:S23-S27.
- Clancy RR, Legido A. (1987) The exact ictal and interictal duration of electroencephalographic neonatal seizures. *Epilepsia* 28:537-541.
- Clancy RR, Legido A. (1991) Postnatal epilepsy after EEG-confirmed neonatal seizures. *Epilepsia* 32:69-76.
- Clancy RR, Legido A, Lewis D. (1988) Occult neonatal seizures. *Epilepsia* 29:256-261.
- Clark AM, Kriel RL, Leppik IE, White JR, Henry TR, Brundage RC, Cloyd JC. (2013) Intravenous topiramate: safety and pharmacokinetics following a single dose in patients with epilepsy or migraines taking oral topiramate. *Epilepsia* 54:1106-1111.
- Cleary RT, Sun H, Huynh T, Manning SM, Li Y, Rotenberg A, Talos DM, Kahle KT, Jackson M, Rakhade SN, Berry G, Jensen FE. (2013) Bumetanide enhances phenobarbital efficacy in a rat model of hypoxic neonatal seizures. *PLoS One* 8:e57148.
- Clynen E, Swijsen A, Raijmakers M, Hoogland G, Rigo JM. (2014) Neuropeptides as targets for the development of anticonvulsant drugs. *Mol Neurobiol* 50:626-646.
- Coker SB, Beltran RS, Myers TF, Hmura L. (1988) Neonatal stroke: description of patients and investigation into pathogenesis. *Pediatr Neurol* 4:219-223.
- Connell J, Oozeer R, de VL, Dubowitz LM, Dubowitz V. (1989) Clinical and EEG response to anticonvulsants in neonatal seizures. *Arch Dis Child* 64:459-464.
- Cornejo BJ, Mesches MH, Coultrap S, Browning MD, Benke TA. (2007) A single episode of neonatal seizures permanently alters glutamatergic synapses. *Ann Neurol* 61:411-426.
- Cowan F, Mercuri E, Groenendaal F, Bassi L, Ricci D, Rutherford M, de VL. (2005) Does cranial ultrasound imaging identify arterial cerebral infarction in term neonates? *Arch Dis Child Fetal Neonatal Ed* 90:F252-F256.
- Cowan F, Rutherford M, Groenendaal F, Eken P, Mercuri E, Bydder GM, Meiners LC, Dubowitz LM, de Vries LS. (2003) Origin and timing of brain lesions in term infants with neonatal encephalopathy. *Lancet* 361:736-742.
- Cross JH. (2014) Setting the scene: definition of prolonged seizures, acute repetitive seizures, and status epilepticus. Do we know why seizures stop? *Epileptic Disord* 16 Suppl 1:2-5.
- Danner R, Shewmon DA, Sherman MP. (1985) Seizures in an atelencephalic infant. Is the cortex essential for neonatal seizures? *Arch Neurol* 42:1014-1016.
- Davidson JO, Yuill CA, Wassink G, Bennet L, Gunn AJ. (2015) Spontaneous pre-existing hypoxia does not affect brain damage after global cerebral ischaemia in late-gestation fetal sheep. *Dev Neurosci* 37:56-65.
- de Vries LS, Groenendaal F, Eken P, van H, I, Rademaker KJ, Meiners LC. (1997) Infarcts in the vascular distribution of the middle cerebral artery in preterm and fullterm infants. *Neuropediatrics* 28:88-96.
- de Vries LS, Hellstrom-Westas L. (2005) Role of cerebral function monitoring in the newborn. *Arch Dis Child Fetal Neonatal Ed* 90:F201-F207.
- de Vries LS, Toet MC. (2006) Amplitude integrated electroencephalography in the full-term newborn. *Clin Perinatol* 33:619-32, vi.
- Deburchgraeve W, Cherian PJ, De VM, Swarte RM, Blok JH, Visser GH, Govaert P, Van HS. (2008) Automated neonatal seizure detection mimicking a human observer reading EEG. *Clin Neurophysiol* 119:2447-2454.
- DeLorenzo RJ, Garnett LK, Towne AR, Waterhouse EJ, Boggs JG, Morton L, Choudhry MA, Barnes T, Ko D. (1999) Comparison of status epilepticus with prolonged seizure episodes lasting from 10 to 29 minutes. *Epilepsia* 40:164-169.
- DeLorenzo RJ, Kirmani B, Deshpande LS, Jakkampudi V, Towne AR, Waterhouse E, Garnett L, Ramakrishnan V. (2009) Comparisons of the mortality and clinical presentations of status epilepticus in private practice community and university hospital settings in Richmond, Virginia. *Seizure* 18:405-411.

- Demarque M, Villeneuve N, Manent JB, Becq H, Represa A, Ben-Ari Y, Aniksztejn L. (2004) Glutamate transporters prevent the generation of seizures in the developing rat neocortex. *J Neurosci* 24:3289-3294.
- Dennis M, Spiegler BJ, Juraneck JJ, Bigler ED, Snead OC, Fletcher JM. (2013) Age, plasticity, and homeostasis in childhood brain disorders. *Neurosci Biobehav Rev*.
- DeVeber G, Andrew M, Adams C, Bjornson B, Booth F, Buckley DJ, Camfield CS, David M, Humphreys P, Langevin P, MacDonald EA, Gillett J, Meaney B, Shevell M, Sinclair DB, Yager J. (2001) Cerebral sinovenous thrombosis in children. *N Engl J Med* 345:417-423.
- Dingley J, Tooley J, Liu X, Scull-Brown E, Elstad M, Chakkarapani E, Sabir H, Thoresen M. (2014) Xenon ventilation during therapeutic hypothermia in neonatal encephalopathy: a feasibility study. *Pediatrics*.
- Dobolyi A, Kekesi KA, Juhasz G, Szekely AD, Lovas G, Kovacs Z. (2014) Receptors of peptides as therapeutic targets in epilepsy research. *Curr Med Chem* 21:764-787.
- Dreissen YE, Bakker MJ, Koelman JH, Tijssen MA. (2012) Exaggerated startle reactions. *Clin Neurophysiol* 123:34-44.
- Dzhala V, Valeeva G, Glykys J, Khazipov R, Staley K. (2012) Traumatic alterations in GABA signaling disrupt hippocampal network activity in the developing brain. *J Neurosci* 32:4017-4031.
- Dzhala VI, Brumback AC, Staley KJ. (2008) Bumetanide enhances phenobarbital efficacy in a neonatal seizure model. *Ann Neurol* 63:222-235.
- Dzhala VI, Kuchibhotla KV, Glykys JC, Kahle KT, Swiercz WB, Feng G, Kuner T, Augustine GJ, Bacskai BJ, Staley KJ. (2010) Progressive NKCC1-dependent neuronal chloride accumulation during neonatal seizures. *J Neurosci* 30:11745-11761.
- Dzhala VI, Talos DM, Sdrulla DA, Brumback AC, Mathews GC, Benke TA, Delpire E, Jensen FE, Staley KJ. (2005) NKCC1 transporter facilitates seizures in the developing brain. *Nat Med* 11:1205-1213.
- Eaton MM, Lim YB, Bracamontes J, Steinbach JH, Akk G. (2012) Agonist-specific conformational changes in the alpha1-gamma2 subunit interface of the GABA A receptor. *Mol Pharmacol* 82:255-263.
- Edwards AD, Brocklehurst P, Gunn AJ, Halliday H, Juszczak E, Levene M, Strohm B, Thoresen M, Whitelaw A, Azzopardi D. (2010) Neurological outcomes at 18 months of age after moderate hypothermia for perinatal hypoxic ischaemic encephalopathy: synthesis and meta-analysis of trial data. *BMJ* 340:c363.
- Estan J, Hope P. (1997) Unilateral neonatal cerebral infarction in full term infants. *Arch Dis Child Fetal Neonatal Ed* 76:F88-F93.
- Evans DJ, Levene M, Rennie JM, Robertson NRC, 1999. Hypoxic-ischemic injury. Textbook of Neonatology. Churchill Livingstone, Edinburgh, pp. 1231-1251.
- Faught E. (2001) Pharmacokinetic considerations in prescribing antiepileptic drugs. *Epilepsia* 42 Suppl 4:19-23.
- Faulkner S, Bainbridge A, Kato T, Chandrasekaran M, Kapetanakis AB, Hristova M, Liu M, Evans S, De VE, Kelen D, Sanders RD, Edwards AD, Maze M, Cady EB, Raivich G, Robertson NJ. (2011) Xenon augmented hypothermia reduces early lactate/N-acetylaspartate and cell death in perinatal asphyxia. *Ann Neurol* 70:133-150.
- Fejerman N. (2005) Nonepileptic disorders imitating generalized idiopathic epilepsies. *Epilepsia* 46 Suppl 9:80-83.
- Fenichel GM, Olson BJ, Fitzpatrick JE. (1980) Heart rate changes in convulsive and nonconvulsive neonatal apnea. *Ann Neurol* 7:577-582.
- Ferriero DM. (2004) Neonatal brain injury. *N Engl J Med* 351:1985-1995.
- Filan PM, Inder TE, Anderson PJ, Doyle LW, Hunt RW. (2007) Monitoring the neonatal brain: a survey of current practice among Australian and New Zealand neonatologists. *J Paediatr Child Health* 43:557-559.
- Filipek PA, Krishnamoorthy KS, Davis KR, Kuehnle K. (1987) Focal cerebral infarction in the newborn: a distinct entity. *Pediatr Neurol* 3:141-147.
- Filippi L, la MG, Cavallaro G, Fiorini P, Favelli F, Malvagia S, Donzelli G, Guerrini R. (2011) Phenobarbital for neonatal seizures in hypoxic ischemic encephalopathy: a pharmacokinetic study during whole body hypothermia. *Epilepsia* 52:794-801.
- Floyer J, 1674. Of Cold Baths. Edward Baynard (Ed.) Psychrolousia. Or, the History of Cold Bathing: Both Ancient and Modern., 5th Ed. William and John Innys, London, pp. 76.
- Folbergrova J, Memezawa H, Smith ML, Siesjo BK. (1992) Focal and perifocal changes in tissue energy state during middle cerebral artery occlusion in normo- and hyperglycemic rats. *J Cereb Blood Flow Metab* 12:25-33.

- Forcelli PA, Janssen MJ, Vicini S, Gale K. (2012) Neonatal exposure to antiepileptic drugs disrupts striatal synaptic development. *Ann Neurol* 72:363-372.
- Friedman L, Hu S. (2014) Early-life seizures in predisposing neuronal preconditioning: a critical review. *Life Sci* 94:92-98.
- Fujikawa DG. (2005) Prolonged seizures and cellular injury: understanding the connection. *Epilepsy Behav* 7 Suppl 3:S3-11.
- Fujimoto S, Yokochi K, Togari H, Nishimura Y, Inukai K, Futamura M, Sobajima H, Suzuki S, Wada Y. (1992) Neonatal cerebral infarction: symptoms, CT findings and prognosis. *Brain Dev* 14:48-52.
- Garfinkle J, Shevell MI. (2011) Predictors of outcome in term infants with neonatal seizures subsequent to intrapartum asphyxia. *J Child Neurol* 26:453-459.
- Gavilanes AW, Vles JS, von SK, van SR, Reulen JP, Blanco CE. (2004) Neonatal electrocortical brain activity and cerebral tissue oxygenation during non-acidotic, normocarbic and normotensive graded hypoxemia. *Clin Neurophysiol* 115:282-288.
- Gerrits LC, Battin MR, Bennet L, Gonzalez H, Gunn AJ. (2005) Epileptiform activity during rewarming from moderate cerebral hypothermia in the near-term fetal sheep. *Pediatr Res* 57:342-346.
- Gillam-Krakauer M, Carter BS. (2012) Neonatal hypoxia and seizures. *Pediatr Rev* 33:387-396.
- Gilman JT, Gal P, Duchowny MS, Weaver RL, Ransom JL. (1989) Rapid sequential phenobarbital treatment of neonatal seizures. *Pediatrics* 83:674-678.
- Glass HC. (2014) Neonatal seizures: advances in mechanisms and management. *Clin Perinatol* 41:177-190.
- Glass HC, Bonifacio SL, Peloquin S, Shimotake T, Sehring S, Sun Y, Sullivan J, Rogers E, Barkovich AJ, Rowitch D, Ferriero DM. (2010) Neurocritical care for neonates. *Neurocrit Care* 12:421-429.
- Glass HC, Glidden D, Jeremy RJ, Barkovich AJ, Ferriero DM, Miller SP. (2009) Clinical neonatal seizures are independently associated with outcome in infants at risk for hypoxic-ischemic brain injury. *J Pediatr* 155:318-323.
- Glass HC, Hong KJ, Rogers EE, Jeremy RJ, Bonifacio SL, Sullivan JE, Barkovich AJ, Ferriero DM. (2011a) Risk factors for epilepsy in children with neonatal encephalopathy. *Pediatr Res* 70:535-540.
- Glass HC, Kan J, Bonifacio SL, Ferriero DM. (2012) Neonatal seizures: treatment practices among term and preterm infants. *Pediatr Neurol* 46:111-115.
- Glass HC, Nash KB, Bonifacio SL, Barkovich AJ, Ferriero DM, Sullivan JE, Cilio MR. (2011b) Seizures and magnetic resonance imaging-detected brain injury in newborns cooled for hypoxic-ischemic encephalopathy. *J Pediatr* 159:731-735.
- Glass HC, Poulin C, Shevell MI. (2011c) Topiramate for the treatment of neonatal seizures. *Pediatr Neurol* 44:439-442.
- Glass HC, Wirrell E. (2009) Controversies in neonatal seizure management. *J Child Neurol* 24:591-599.
- Glass HC, Wusthoff CJ, Shellhaas RA. (2013) Amplitude-integrated electro-encephalography: the child neurologist's perspective. *J Child Neurol* 28:1342-1350.
- Glass HC, Wusthoff CJ, Shellhaas RA, Tsuchida TN, Bonifacio SL, Cordeiro M, Sullivan J, Abend NS, Chang T. (2014) Risk factors for EEG seizures in neonates treated with hypothermia: a multicenter cohort study. *Neurology* 82:1239-1244.
- Globus MY, Alonso O, Dietrich WD, Busto R, Ginsberg MD. (1995) Glutamate release and free radical production following brain injury: effects of posttraumatic hypothermia. *J Neurochem* 65:1704-1711.
- Gluckman PD, Williams CE. (1992) When and why do brain cells die? *Dev Med Child Neurol* 34:1010-1014.
- Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, Polin RA, Robertson CM, Thoresen M, Whitelaw A, Gunn AJ. (2005) Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet* 365:663-670.
- Glykys J, Dzhalal VI, Kuchibhotla KV, Feng G, Kuner T, Augustine G, Bacskaï BJ, Staley KJ. (2009) Differences in cortical versus subcortical GABAergic signaling: a candidate mechanism of electroclinical uncoupling of neonatal seizures. *Neuron* 63:657-672.
- Golomb MR, Garg BP, Carvalho KS, Johnson CS, Williams LS. (2007) Perinatal stroke and the risk of developing childhood epilepsy. *J Pediatr* 151:409-413.
- Gotman J. (1982) Automatic recognition of epileptic seizures in the EEG. *Electroencephalogr Clin Neurophysiol* 54:530-540.

- Gotman J, Flanagan D, Rosenblatt B, Bye A, Mizrahi EM. (1997a) Evaluation of an automatic seizure detection method for the newborn EEG. *Electroencephalogr Clin Neurophysiol* 103:363-369.
- Gotman J, Flanagan D, Zhang J, Rosenblatt B. (1997b) Automatic seizure detection in the newborn: methods and initial evaluation. *Electroencephalogr Clin Neurophysiol* 103:356-362.
- Govaert P, Matthys E, Zecic A, Roelens F, Oostra A, Vanzielegheem B. (2000) Perinatal cortical infarction within middle cerebral artery trunks. *Arch Dis Child Fetal Neonatal Ed* 82:F59-F63.
- Govaert P, Ramenghi L, Taal R, Dudink J, Lequin M. (2009a) Diagnosis of perinatal stroke II: mechanisms and clinical phenotypes. *Acta Paediatr* 98:1720-1726.
- Govaert P, Smith L, Dudink J. (2009b) Diagnostic management of neonatal stroke. *Semin Fetal Neonatal Med* 14:323-328.
- Guillet R, Kwon J. (2007) Seizure recurrence and developmental disabilities after neonatal seizures: outcomes are unrelated to use of phenobarbital prophylaxis. *J Child Neurol* 22:389-395.
- Gunn AJ, Battin M, Gluckman PD, Gunn TR, Bennet L. (2005) Therapeutic hypothermia: from lab to NICU. *J Perinat Med* 33:340-346.
- Gunn AJ, Gunn TR, De Haan HH, Williams CE, Gluckman PD. (1997) Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal lambs. *J Clin Invest* 99:248-256.
- Gunn AJ, Parer JT, Mallard EC, Williams CE, Gluckman PD. (1992) Cerebral histologic and electrocorticographic changes after asphyxia in fetal sheep. *Pediatr Res* 31:486-491.
- Gunther G, Junker R, Strater R, Schobess R, Kurnik K, Heller C, Kosch A, Nowak-Gottl U. (2000) Symptomatic ischemic stroke in full-term neonates : role of acquired and genetic prothrombotic risk factors. *Stroke* 31:2437-2441.
- Gupta N, Pappas A, Thomas R, Shankaran S. (2015) Reference Values for Three Channels of Amplitude-Integrated EEG Using the Brainz BRM3 Cerebral Function Monitor in Normal Term Neonates: A Pilot Study. *Pediatr Neurol* 52:344-348.
- Gutherz SB, Kulick CV, Soper C, Kondratyev A, Gale K, Forcelli PA. (2014) Brief postnatal exposure to phenobarbital impairs passive avoidance learning and sensorimotor gating in rats. *Epilepsy Behav* 37:265-269.
- Hamelin S, Delnard N, Cneude F, Debillon T, Vercueil L. (2011) Influence of hypothermia on the prognostic value of early EEG in full-term neonates with hypoxic ischemic encephalopathy. *Neurophysiol Clin* 41:19-27.
- Harbert MJ, Tam EW, Glass HC, Bonifacio SL, Haeusslein LA, Barkovich AJ, Jeremy RJ, Rogers EE, Glidden DV, Ferriero DM. (2011) Hypothermia is correlated with seizure absence in perinatal stroke. *J Child Neurol* 26:1126-1130.
- Harteman JC, Groenendaal F, Kwee A, Welsing PM, Benders MJ, de Vries LS. (2012) Risk factors for perinatal arterial ischaemic stroke in full-term infants: a case-control study. *Arch Dis Child Fetal Neonatal Ed* 97:F411-F416.
- Hebb DO. (1967) Cerebral organization and consciousness. *Res Publ Assoc Res Nerv Ment Dis* 45:1-7.
- Heiss WD, Huber M, Fink GR, Herholz K, Pietrzyk U, Wagner R, Wienhard K. (1992) Progressive derangement of periinfarct viable tissue in ischemic stroke. *J Cereb Blood Flow Metab* 12:193-203.
- Hellström-Westas L, Ingmar R. 2003. An Atlas of Amplitude-integrated EEGs in the Newborn Encyclopedia of visual medicine series. Parthenon Publishing Group.
- Hellstrom-Westas L. (2008) Amplitude-integrated EEG--useful for early outcome prediction after birth asphyxia? *Nat Clin Pract Neurol* 4:74-75.
- Hellstrom-Westas L, Boylan G, Agren J. (2015) Systematic review of neonatal seizure management strategies provides guidance on anti-epileptic treatment. *Acta Paediatr* 104:123-129.
- Hellstrom-Westas L, Rosen I. (2006) Continuous brain-function monitoring: state of the art in clinical practice. *Semin Fetal Neonatal Med* 11:503-511.
- Herrera EA, Macchiavello R, Montt C, Ebensperger G, Diaz M, Ramirez S, Parer JT, Seron-Ferre M, Reyes RV, Llanos AJ. (2014) Melatonin improves cerebrovascular function and decreases oxidative stress in chronically hypoxic lambs. *J Pineal Res*.
- Hill MW, Wong M, Amarakone A, Rothman SM. (2000) Rapid cooling aborts seizure-like activity in rodent hippocampal-entorhinal slices. *Epilepsia* 41:1241-1248.
- Holcroft CJ, Askin FB, Patra A, Allen MC, Blakemore KJ, Graham EM. (2004) Are histopathologic chorioamnionitis and funisitis associated with metabolic acidosis in the preterm fetus? *Am J Obstet Gynecol* 191:2010-2015.

- Holmes GL. (1997) Epilepsy in the developing brain: lessons from the laboratory and clinic. *Epilepsia* 38:12-30.
- Holmes GL, Ben-Ari Y. (2001) The neurobiology and consequences of epilepsy in the developing brain. *Pediatr Res* 49:320-325.
- Holmes GL, Ben-Ari Y. (2003) Seizing hold of seizures. *Nat Med* 9:994-996.
- Hoogstraate SR, Lequin MH, Huysman MA, Ahmed S, Govaert PP. (2009) Apnoea in relation to neonatal temporal lobe haemorrhage. *Eur J Paediatr Neurol* 13:356-361.
- Hu KC, Chiu NC, Ho CS, Lee ST, Shen EY. (2003) Continuous midazolam infusion in the treatment of uncontrollable neonatal seizures. *Acta Paediatr Taiwan* 44:279-281.
- Huang T, Huang W, Zhang Z, Yu L, Xie C, Zhu D, Peng Z, Chen J. (2014) Hypoxia-inducible factor-1alpha upregulation in microglia following hypoxia protects against ischemia-induced cerebral infarction. *Neuroreport* 25:1122-1128.
- Isaev D, Isaeva E, Khazipov R, Holmes GL. (2007) Shunting and hyperpolarizing GABAergic inhibition in the high-potassium model of ictogenesis in the developing rat hippocampus. *Hippocampus* 17:210-219.
- Jackson JH. (1890) The Lumleian Lectures on Convulsive Seizures. *Br Med J* 1:821-827.
- Jensen FE. (2006) Developmental factors regulating susceptibility to perinatal brain injury and seizures. *Curr Opin Pediatr* 18:628-633.
- Jensen FE. (2009a) Developmental factors in the pathogenesis of neonatal seizures. *J Pediatr Neurol* 7:5-12.
- Jensen FE. (2009b) Neonatal seizures: an update on mechanisms and management. *Clin Perinatol* 36:881-900.
- Jiang Q, Wang J, Wu X, Jiang Y. (2007) Alterations of NR2B and PSD-95 expression after early-life epileptiform discharges in developing neurons. *Int J Dev Neurosci* 25:165-170.
- Jin G, Liu B, You Z, Bambakidis T, Dekker SE, Maxwell J, Halaweish I, Linzel D, Alam HB. (2014) Development of a novel neuroprotective strategy: combined treatment with hypothermia and valproic acid improves survival in hypoxic hippocampal cells. *Surgery* 156:221-228.
- Johnston MV, Fatemi A, Wilson MA, Northington F. (2011) Treatment advances in neonatal neuroprotection and neurointensive care. *Lancet Neurol* 10:372-382.
- Johnston MV, Trescher WH, Ishida A, Nakajima W. (2001) Neurobiology of hypoxic-ischemic injury in the developing brain. *Pediatr Res* 49:735-741.
- Jones AW, Dooley JD, Murphy JR.. (1950) Treatment of choice in barbiturate poisoning; series of twenty-nine cases of barbiturate poisoning treated with pentylenetetrazole (Metrazol) and supportive therapy. *J Am Med Assoc* 143:884-888.
- Jones DM, Esmail N, Maren S, Macdonald RL. (2002) Characterization of pharmacoresistance to benzodiazepines in the rat Li-pilocarpine model of status epilepticus. *Epilepsy Res* 50:301-312.
- Jonsson M, Agren J, Norden-Lindeberg S, Ohlin A, Hanson U. (2014) Suboptimal care and metabolic acidemia is associated with neonatal encephalopathy but not with neonatal seizures alone: a population-based clinical audit. *Acta Obstet Gynecol Scand* 93:477-482.
- Kaddurah AK, Holmes GL. (2009) Benign neonatal sleep myoclonus: history and semiology. *Pediatr Neurol* 40:343-346.
- Kahle KT, Staley KJ. (2012) Neonatal Seizures and Neuronal Transmembrane Ion Transport.
- Kendall GS, Mathieson S, Meek J, Rennie JM. (2012) Recooling for rebound seizures after rewarming in neonatal encephalopathy. *Pediatrics* 130:e451-e455.
- Khanna A, Walcott BP, Kahle KT. (2013) Limitations of Current GABA Agonists in Neonatal Seizures: Toward GABA Modulation Via the Targeting of Neuronal Cl(-) Transport. *Front Neurol* 4:78.
- Khazipov R, Khalilov I, Tyzio R, Morozova E, Ben-Ari Y, Holmes GL. (2004) Developmental changes in GABAergic actions and seizure susceptibility in the rat hippocampus. *Eur J Neurosci* 19:590-600.
- Kim JS, Kondratyev A, Tomita Y, Gale K. (2007) Neurodevelopmental impact of antiepileptic drugs and seizures in the immature brain. *Epilepsia* 48 Suppl 5:19-26.
- Kirton A, rmstrong-Wells J, Chang T, Deveber G, Rivkin MJ, Hernandez M, Carpenter J, Yager JY, Lynch JK, Ferriero DM. (2011) Symptomatic neonatal arterial ischemic stroke: the International Pediatric Stroke Study. *Pediatrics* 128:e1402-e1410.
- Klem GH, Luders HO, Jasper HH, Elger C. (1999) The ten-twenty electrode system of the International Federation. The International Federation of Clinical Neurophysiology. *Electroencephalogr Clin Neurophysiol Suppl* 52:3-6.

- Klitgaard H, Matagne A, Vanneste-Goemaere J, Margineanu DG. (2002) Pilocarpine-induced epileptogenesis in the rat: impact of initial duration of status epilepticus on electrophysiological and neuropathological alterations. *Epilepsy Res* 51:93-107.
- Koh S, Jensen FE. (2001) Topiramate blocks perinatal hypoxia-induced seizures in rat pups. *Ann Neurol* 50:366-372.
- Kohelet D, Shochat R, Lusky A, Reichman B. (2004) Risk factors for neonatal seizures in very low birthweight infants: population-based survey. *J Child Neurol* 19:123-128.
- Korosi A, Shanabrough M, McClelland S, Liu ZW, Borok E, Gao XB, Horvath TL, Baram TZ. (2010) Early-life experience reduces excitation to stress-responsive hypothalamic neurons and reprograms the expression of corticotropin-releasing hormone. *J Neurosci* 30:703-713.
- Kovac S, Abramov AY, Walker MC. (2013) Energy depletion in seizures: anaplerosis as a strategy for future therapies. *Neuropharmacology* 69:96-104.
- Kristian T. (2004) Metabolic stages, mitochondria and calcium in hypoxic/ischemic brain damage. *Cell Calcium* 36:221-233.
- Kwon JM, Guillet R, Shankaran S, Laptook AR, McDonald SA, Ehrenkranz RA, Tyson JE, O'Shea TM, Goldberg RN, Donovan EF, Fanaroff AA, Poole WK, Higgins RD, Walsh MC. (2011) Clinical seizures in neonatal hypoxic-ischemic encephalopathy have no independent impact on neurodevelopmental outcome: secondary analyses of data from the neonatal research network hypothermia trial. *J Child Neurol* 26:322-328.
- Lado FA, Laureta EC, Moshe SL. (2002) Seizure-induced hippocampal damage in the mature and immature brain. *Epileptic Disord* 4:83-97.
- Lado FA, Moshe SL. (2008) How do seizures stop? *Epilepsia* 49:1651-1664.
- Lai MC, Yang SN. (2011) Perinatal hypoxic-ischemic encephalopathy. *J Biomed Biotechnol* 2011:609813.
- Lamblin MD, Walls EE, Andre M. (2013) The electroencephalogram of the full-term newborn: review of normal features and hypoxic-ischemic encephalopathy patterns. *Neurophysiol Clin* 43:267-287.
- Lawn J, Shibuya K, Stein C. (2005a) No cry at birth: global estimates of intrapartum stillbirths and intrapartum-related neonatal deaths. *Bull World Health Organ* 83:409-417.
- Lawn JE, Cousens S, Zupan J. (2005b) 4 million neonatal deaths: when? Where? Why? *Lancet* 365:891-900.
- Lawn JE, Kerber K, Enweronu-Laryea C, Cousens S. (2010) 3.6 million neonatal deaths--what is progressing and what is not? *Semin Perinatol* 34:371-386.
- Lawrence R, Inder T. (2010) Neonatal status epilepticus. *Semin Pediatr Neurol* 17:163-168.
- Lawrence R, Mathur A, Nguyen The Tich, Zempel J, Inder T. (2009) A pilot study of continuous limited-channel aEEG in term infants with encephalopathy. *J Pediatr* 154:835-841.
- Levene ML, Kornberg J, Williams TH. (1985) The incidence and severity of post-asphyxial encephalopathy in full-term infants. *Early Hum Dev* 11:21-26.
- Levy SR, Abrams IF, Marshall PC, Rosquete EE. (1985) Seizures and cerebral infarction in the full-term newborn. *Ann Neurol* 17:366-370.
- Liu A, Hahn JS, Heldt GP, Coen RW. (1992) Detection of neonatal seizures through computerized EEG analysis. *Electroencephalogr Clin Neurophysiol* 82:30-37.
- Liu Y, Barks JD, Xu G, Silverstein FS. (2004) Topiramate extends the therapeutic window for hypothermia-mediated neuroprotection after stroke in neonatal rats. *Stroke* 35:1460-1465.
- Lobo N, Yang B, Rizvi M, Ma D. (2013) Hypothermia and xenon: novel noble guardians in hypoxic-ischemic encephalopathy? *J Neurosci Res* 91:473-478.
- Loiacono G, Masci M, Zaccara G, Verrotti A. (2014) The treatment of neonatal seizures: focus on Levetiracetam. *J Matern Fetal Neonatal Med* 1-6.
- Loomis AL, Harvey EN, Hobart G. (1936) Electrical potentials of the human brain. *Journal of Experimental Psychology* 19:249-279.
- Lorek A, Takei Y, Cady EB, Wyatt JS, Penrice J, Edwards AD, Peebles D, Wylezinska M, Owen-Reece H, Kirkbride V, .. (1994) Delayed ("secondary") cerebral energy failure after acute hypoxia-ischemia in the newborn piglet: continuous 48-hour studies by phosphorus magnetic resonance spectroscopy. *Pediatr Res* 36:699-706.

- Loscher W, Rogawski MA. (2012) How theories evolved concerning the mechanism of action of barbiturates. *Epilepsia* 53 Suppl 8:12-25.
- Low E, Boylan GB, Mathieson SR, Murray DM, Korotchikova I, Stevenson NJ, Livingstone V, Rennie JM. (2012a) Cooling and seizure burden in term neonates: an observational study. *Arch Dis Child Fetal Neonatal Ed* 97:F267-F272.
- Low E, Dempsey EM, Ryan CA, Rennie JM, Boylan GB. (2012b) EEG suppression associated with apneic episodes in a neonate. *Case Rep Neurol Med* 2012:250801.
- Low E, Mathieson SR, Stevenson NJ, Livingstone V, Ryan CA, Bogue CO, Rennie JM, Boylan GB. (2014) Early postnatal EEG features of perinatal arterial ischaemic stroke with seizures. *PLoS One* 9:e100973.
- Lundqvist M, Agren J, Hellstrom-Westas L, Flink R, Wickstrom R. (2013) Efficacy and safety of lidocaine for treatment of neonatal seizures. *Acta Paediatr* 102:863-867.
- Lynch N, Low E, Rennie JM, and Boylan GB. Comparison of seizure characteristics in full term neonates with stroke and hypoxic-ischemic encephalopathy. *Archives of Diseases in Childhood* 96, A39.doi:10.1136/adc.2011.212563.84. 2011. Ref Type: Abstract
- Lynch JK, Hirtz DG, Deveber G, Nelson KB. (2002) Report of the National Institute of Neurological Disorders and Stroke workshop on perinatal and childhood stroke. *Pediatrics* 109:116-123.
- Lynch JK, Nelson KB. (2001) Epidemiology of perinatal stroke. *Curr Opin Pediatr* 13:499-505.
- Lynch NE, Stevenson NJ, Livingstone V, Murphy BP, Rennie JM, Boylan GB. (2012) The temporal evolution of electrographic seizure burden in neonatal hypoxic ischemic encephalopathy. *Epilepsia* 53:549-557.
- Maartens IA, Wassenberg T, Buijs J, Bok L, de Kleine MJ, Katgert T, Andriessen P. (2012) Neurodevelopmental outcome in full-term newborns with refractory neonatal seizures. *Acta Paediatr* 101:e173-e178.
- Malik SI, Painter MJ, Venkataramanan R, Alvin JD. (2003) Phenytoin and phenobarbital stable isotope studies in neonates. *Pediatr Neurol* 29:376-380.
- Malingre MM, van Rooij LG, Rademaker CM, Toet MC, Ververs TF, van KC, de Vries LS. (2006) Development of an optimal lidocaine infusion strategy for neonatal seizures. *Eur J Pediatr* 165:598-604.
- Mallard EC, Waldvogel HJ, Williams CE, Faull RL, Gluckman PD. (1995) Repeated asphyxia causes loss of striatal projection neurons in the fetal sheep brain. *Neuroscience* 65:827-836.
- Malone A, Ryan CA, Fitzgerald A, Burgoyne L, Connolly S, Boylan GB. (2009) Interobserver agreement in neonatal seizure identification. *Epilepsia* 50:2097-2101.
- Manthey D, Asimiadou S, Stefovskia V, Kaindl AM, Fassbender J, Ikonomidou C, Bittigau P. (2005) Sulthiame but not levetiracetam exerts neurotoxic effect in the developing rat brain. *Exp Neurol* 193:497-503.
- Marks MP, Holmgren EB, Fox AJ, Patel S, von KR, Froehlich J. (1999) Evaluation of early computed tomographic findings in acute ischemic stroke. *Stroke* 30:389-392.
- Marlow N, Budge H. (2005) Prevalence, causes, and outcome at 2 years of age of newborn encephalopathy. *Arch Dis Child Fetal Neonatal Ed* 90:F193-F194.
- Marret S, Lardennois C, Mercier A, Radi S, Michel C, Vanhulle C, Charollais A, Gressens P. (2001) Fetal and neonatal cerebral infarcts. *Biol Neonate* 79:236-240.
- Marsot A, Brevaut-Malaty V, Vialet R, Boulamery A, Bruguerolle B, Simon N. (2013) Pharmacokinetics and absolute bioavailability of phenobarbital in neonates and young infants, a population pharmacokinetic modelling approach. *Fundam Clin Pharmacol* doi: 10.1111/fcp.12042.
- Mathers DA, Wan X, Puil E. (2007) Barbiturate activation and modulation of GABA(A) receptors in neocortex. *Neuropharmacology* 52:1160-1168.
- Mathieson SR, Livingstone VL, Low E, Renie JM, and Boylan GB. Reduction in EEG Amplitude and Propagation of Neonatal Seizures after Phenobarbitone May Explain Electroclinical Dissociation. *Proceedings from the Pediatric Academy Societies* . 2014. Ref Type: Abstract
- Maurer VO, Rizzi M, Bianchetti MG, Ramelli GP. (2010) Benign neonatal sleep myoclonus: a review of the literature. *Pediatrics* 125:e919-e924.
- Maynard D, Prior PF, Scott DF. (1969) Device for continuous monitoring of cerebral activity in resuscitated patients. *Br Med J* 4:545-546.

- McBride MC, Laroia N, Guillet R. (2000) Electrographic seizures in neonates correlate with poor neurodevelopmental outcome. *Neurology* 55:506-513.
- Memezawa H, Minamisawa H, Smith ML, Siesjo BK. (1992) Ischemic penumbra in a model of reversible middle cerebral artery occlusion in the rat. *Exp Brain Res* 89:67-78.
- Mendoza JC, Shearer LL, Cook LN. (1991) Lateralization of brain lesions following extracorporeal membrane oxygenation. *Pediatrics* 88:1004-1009.
- Ment LR, Duncan CC, Ehrenkranz RA. (1984) Perinatal cerebral infarction. *Ann Neurol* 16:559-568.
- Mercado J, Czajkowski C. (2008) Gamma-aminobutyric acid (GABA) and pentobarbital induce different conformational rearrangements in the GABA A receptor alpha1 and beta2 pre-M1 regions. *J Biol Chem* 283:15250-15257.
- Mercuri E. (2001) Early diagnostic and prognostic indicators in full term infants with neonatal cerebral infarction: an integrated clinical, neuroradiological and EEG approach. *Minerva Pediatr* 53:305-311.
- Mercuri E, Cowan F, Rutherford M, Acolet D, Pennock J, Dubowitz L. (1995) Ischaemic and haemorrhagic brain lesions in newborns with seizures and normal Apgar scores. *Arch Dis Child Fetal Neonatal Ed* 73:F67-F74.
- Mercuri E, Rutherford M, Cowan F, Pennock J, Counsell S, Papadimitriou M, Azzopardi D, Bydder G, Dubowitz L. (1999) Early prognostic indicators of outcome in infants with neonatal cerebral infarction: a clinical, electroencephalogram, and magnetic resonance imaging study. *Pediatrics* 103:39-46.
- Messer J, Haddad J, Casanova R. (1991) Transcranial Doppler evaluation of cerebral infarction in the neonate. *Neuropediatrics* 22:147-151.
- Mikati MA, Werner S, Shalak L, Shamseddine A, Simaan E, Liu Z, Tarif S, Stafstrom C, Holmes G. (2003) Stages of status epilepticus in the developing brain. *Epilepsy Res* 55:9-19.
- Miller JA. Jr, Miller FS., Westin B.. (1964) Hypothermia in the treatment of asphyxia neonatorum. *Biol Neonat* 6:148-163.
- Miller SP, Weiss J, Barnwell A, Ferriero DM, Latal-Hajnal B, Ferrer-Rogers A, Newton N, Partridge JC, Glidden DV, Vigneron DB, Barkovich AJ. (2002) Seizure-associated brain injury in term newborns with perinatal asphyxia. *Neurology* 58:542-548.
- Miller V. (2000) Neonatal cerebral infarction. *Semin Pediatr Neurol* 7:278-288.
- Minlebaev M, Ben-Ari Y, Khazipov R. (2007) Network mechanisms of spindle-burst oscillations in the neonatal rat barrel cortex in vivo. *J Neurophysiol* 97:692-700.
- Mitra J, Glover JR, Ktonas PY, Thitai KA, Mukherjee A, Karayiannis NB, Frost JD, Jr., Hrachovy RA, Mizrahi EM. (2009) A multistage system for the automated detection of epileptic seizures in neonatal electroencephalography. *J Clin Neurophysiol* 26:218-226.
- Miyagawa T, Sotero M, Avellino AM, Kuratani J, Saneto RP, Ellenbogen RG, Ojemann JG. (2007) Apnea caused by mesial temporal lobe mass lesions in infants: report of 3 cases. *J Child Neurol* 22:1079-1083.
- Mizrahi EM, Kellaway P. 1998. *Diagnosis and Management of Neonatal Seizures*. Lippincott-Raven, Philadelphia.
- Mizrahi EM, Kellaway P. (1987) Characterization and classification of neonatal seizures. *Neurology* 37:1837-1844.
- Moshe SL. (1987) Epileptogenesis and the immature brain. *Epilepsia* 28 Suppl 1:S3-15.
- Moshe SL. (2000) Seizures early in life. *Neurology* 55:S15-S20.
- Motamedi GK, Lesser RP, Vicini S. (2013) Therapeutic brain hypothermia, its mechanisms of action, and its prospects as a treatment for epilepsy. *Epilepsia* 54:959-970.
- Mruk AL, Garlitz KL, Leung NR. (2015) Levetiracetam in neonatal seizures: a review. *J Pediatr Pharmacol Ther* 20:76-89.
- Muroi Y, Theusch CM, Czajkowski C, Jackson MB. (2009) Distinct structural changes in the GABAA receptor elicited by pentobarbital and GABA. *Biophys J* 96:499-509.
- Murray DM, Boylan GB, Ali I, Ryan CA, Murphy BP, Connolly S. (2008) Defining the gap between electrographic seizure burden, clinical expression and staff recognition of neonatal seizures. *Arch Dis Child Fetal Neonatal Ed* 93:F187-F191.
- Murray DM, Boylan GB, Ryan CA, Connolly S. (2006a) Early continuous video-EEG in acute near-total intrauterine asphyxia. *Pediatr Neurol* 35:52-56.
- Murray DM, Boylan GB, Ryan CA, Connolly S. (2009) Early EEG findings in hypoxic-ischemic encephalopathy predict outcomes at 2 years. *Pediatrics* 124:e459-e467.

- Murray DM, Ryan CA, Boylan GB, Fitzgerald AP, Connolly S. (2006b) Prediction of seizures in asphyxiated neonates: correlation with continuous video-electroencephalographic monitoring. *Pediatrics* 118:41-46.
- Nakajima W, Ishida A, Lange MS, Gabrielson KL, Wilson MA, Martin LJ, Blue ME, Johnston MV. (2000) Apoptosis has a prolonged role in the neurodegeneration after hypoxic ischemia in the newborn rat. *J Neurosci* 20:7994-8004.
- Nakashima K, Todd MM. (1996) Effects of hypothermia on the rate of excitatory amino acid release after ischemic depolarization. *Stroke* 27:913-918.
- Nardou R, Yamamoto S, Bhar A, Burnashev N, Ben-Ari Y, Khalilov I. (2011a) Phenobarbital but not diazepam reduces AMPA/kainate receptor mediated currents and exerts opposite actions on initial seizures in the neonatal rat hippocampus. *Front Cell Neurosci* 5:16.
- Nardou R, Yamamoto S, Chazal G, Bhar A, Ferrand N, Dulac O, Ben-Ari Y, Khalilov I. (2011b) Neuronal chloride accumulation and excitatory GABA underlie aggravation of neonatal epileptiform activities by phenobarbital. *Brain* 134:987-1002.
- Nash KB, Bonifacio SL, Glass HC, Sullivan JE, Barkovich AJ, Ferriero DM, Cilio MR. (2011) Video-EEG monitoring in newborns with hypoxic-ischemic encephalopathy treated with hypothermia. *Neurology* 76:556-562.
- Natarajan G, Shankaran S, Laptook AR, Pappas A, Bann CM, McDonald SA, Das A, Higgins RD, Hintz SR, Vohr BR. (2013) Apgar scores at 10 min and outcomes at 6-7 years following hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed* 98:F473-F479.
- National Institute for Health and Clinical Excellence (NICE). Controlled cooling to treat newborn babies with brain injury caused by oxygen shortage during birth. Information about NICE procedure guidance. 347; Reference No N2184 [online]. www.nice.org.uk . 2010.
- Navakatikyan MA, Colditz PB, Burke CJ, Inder TE, Richmond J, Williams CE. (2006) Seizure detection algorithm for neonates based on wave-sequence analysis. *Clin Neurophysiol* 117:1190-1203.
- Nedergaard M, Hansen AJ. (1993) Characterization of cortical depolarizations evoked in focal cerebral ischemia. *J Cereb Blood Flow Metab* 13:568-574.
- NEMO study. Treatment of NEonatal seizures with Medication Off-patent: evaluation of efficacy and safety of bumetanide. <http://www.nemo-europe.com/> . 2010.
- Nicolaides P, Appleton RE. (1996) Stroke in children. *Dev Med Child Neurol* 38:172-180.
- Niedermeyer E, da Silva FL. 2004. Electroencephalography: Basic Principles, Clinical Applications and Related Fields, 5th Ed. Lippincott Williams & Wilkins, Philadelphia.
- Nishimaki S, Seki K, Yokota S. (2001) Cerebral blood flow velocity in two patients with neonatal cerebral infarction. *Pediatr Neurol* 24:320-323.
- Northington FJ, Chavez-Valdez R, Martin LJ. (2011) Neuronal cell death in neonatal hypoxia-ischemia. *Ann Neurol* 69:743-758.
- Nunes ML, da Costa JC. (2010) Sleep and epilepsy in neonates. *Sleep Med* 11:665-673.
- Nunes ML, Martins MP, Barea BM, Wainberg RC, Costa JC. (2008) Neurological outcome of newborns with neonatal seizures: a cohort study in a tertiary university hospital. *Arq Neuropsiquiatr* 66:168-174.
- O'Brien FE, Iwata O, Thornton JS, De VE, Sellwood MW, Iwata S, Sakata YS, Charman S, Ordidge R, Cady EB, Wyatt JS, Robertson NJ. (2006) Delayed whole-body cooling to 33 or 35 degrees C and the development of impaired energy generation consequential to transient cerebral hypoxia-ischemia in the newborn piglet. *Pediatrics* 117:1549-1559.
- Oliveira AJ, Nunes ML, Haertel LM, Reis FM, da Costa JC. (2000) Duration of rhythmic EEG patterns in neonates: new evidence for clinical and prognostic significance of brief rhythmic discharges. *Clin Neurophysiol* 111:1646-1653.
- Olsen RW. 2002. Phenobarbital and other barbiturates. Lippincott Williams & Wilkins (Ed.) Antiepileptic drugs, 5th Ed. Philadelphia, PA, pp. 489-495.
- Ortibus EL, Sum JM, Hahn JS. (1996) Predictive value of EEG for outcome and epilepsy following neonatal seizures. *Electroencephalogr Clin Neurophysiol* 98:175-185.
- Pacifici GM. (2014) Clinical pharmacology of midazolam in neonates and children: effect of disease-a review. *Int J Pediatr* 2014:309342.
- Painter MJ, Scher MS, Stein AD, Armatti S, Wang Z, Gardiner JC, Paneth N, Minnigh B, Alvin J. (1999) Phenobarbital compared with phenytoin for the treatment of neonatal seizures. *N Engl J Med* 341:485-489.

- Panayiotopoulos CP. (2004) Autonomic seizures and autonomic status epilepticus peculiar to childhood: diagnosis and management. *Epilepsy Behav* 5:286-295.
- Pape HC. (1996) Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. *Annu Rev Physiol* 58:299-327.
- Paro-Panjan D, Neubauer D. (2008) Benign neonatal sleep myoclonus: experience from the study of 38 infants. *Eur J Paediatr Neurol* 12:14-18.
- Patrizi S, Holmes GL, Orzalesi M, Allemand F. (2003) Neonatal seizures: characteristics of EEG ictal activity in preterm and fullterm infants. *Brain Dev* 25:427-437.
- Payne ET, Zhao XY, Frndova H, McBain K, Sharma R, Hutchison JS, Hahn CD. (2014) Seizure burden is independently associated with short term outcome in critically ill children. *Brain* 137:1429-1438.
- Peng BW, Justice JA, He XH, Sanchez RM. (2013) Decreased A-currents in hippocampal dentate granule cells after seizure-inducing hypoxia in the immature rat. *Epilepsia* 54:1223-1231.
- Perlman JM, Rollins NK, Evans D. (1994) Neonatal stroke: clinical characteristics and cerebral blood flow velocity measurements. *Pediatr Neurol* 11:281-284.
- Pichler G, Cheung PY, Aziz K, Urlsberger B, Schmolzer GM. (2014) How to monitor the brain during immediate neonatal transition and resuscitation? A systematic qualitative review of the literature. *Neonatology* 105:205-210.
- Pinto LC, Giliberti P. (2001) Neonatal seizures: background EEG activity and the electroclinical correlation in full-term neonates with hypoxic-ischemic encephalopathy. Analysis by computer-synchronized long-term polygraphic video-EEG monitoring. *Epileptic Disord* 3:125-132.
- Pisani F, Cerminara C, Fusco C, Sisti L. (2007) Neonatal status epilepticus vs recurrent neonatal seizures: clinical findings and outcome. *Neurology* 69:2177-2185.
- Pisani F, Copioli C, Di GC, Turco E, Sisti L. (2008) Neonatal seizures: relation of ictal video-electroencephalography (EEG) findings with neurodevelopmental outcome. *J Child Neurol* 23:394-398.
- Praveen V, Patole SK, Whitehall JS. (2001) Hyperekplexia in neonates. *Postgrad Med J* 77:570-572.
- Pressler R.M. Neonatal seizures. <http://www.epilepsysociety.org.uk/sites/default/files/attachments/Chapter06Pressler.pdf> . 2015.
- Pressler RM, Boylan GB, Marlow N, Blennow M, Chiron C, Cross JH, de Vries LS, Hallberg B, Hellstrom-Westas L, Jullien V, Livingstone V, Mangum B, Murphy B, Murray D, Pons G, Rennie J, Swarte R, Toet MC, Vanhatalo S, Zohar S. (2015) Bumetanide for the treatment of seizures in newborn babies with hypoxic ischaemic encephalopathy (NEMO): an open-label, dose finding, and feasibility phase 1/2 trial. *Lancet Neurol*.
- Pressler RM, Boylan GB, Morton M, Binnie CD, Rennie JM. (2001) Early serial EEG in hypoxic ischaemic encephalopathy. *Clin Neurophysiol* 112:31-37.
- Pressler RM, Mangum B. (2013) Newly emerging therapies for neonatal seizures. *Semin Fetal Neonatal Med* 18:216-223.
- Puskarjov M, Kahle KT, Ruusuvuori E, Kaila K. (2014) Pharmacotherapeutic targeting of cation-chloride cotransporters in neonatal seizures. *Epilepsia* 55:806-818.
- Rafay MF, Cortez MA, de Veber GA, Tan-Dy C, Al-Futaisi A, Yoon W, Fallah S, Moore AM. (2009) Predictive value of clinical and EEG features in the diagnosis of stroke and hypoxic ischemic encephalopathy in neonates with seizures. *Stroke* 40:2402-2407.
- Rakhade SN, Jensen FE. (2009) Epileptogenesis in the immature brain: emerging mechanisms. *Nat Rev Neurol* 5:380-391.
- Rakhade SN, Klein PM, Huynh T, Hilario-Gomez C, Kosaras B, Rotenberg A, Jensen FE. (2011) Development of later life spontaneous seizures in a rodent model of hypoxia-induced neonatal seizures. *Epilepsia* 52:753-765.
- Rakhade SN, Zhou C, Aujla PK, Fishman R, Sucher NJ, Jensen FE. (2008) Early alterations of AMPA receptors mediate synaptic potentiation induced by neonatal seizures. *J Neurosci* 28:7979-7990.
- Ramenghi LA, Govaert P, Fumagalli M, Bassi L, Mosca F. (2009) Neonatal cerebral sinovenous thrombosis. *Semin Fetal Neonatal Med* 14:278-283.
- Rando T, Ricci D, Mercuri E, Frisone MF, Luciano R, Tortorolo G, Guzzetta F. (2000) Periodic lateralized epileptiform discharges (PLEDs) as early indicator of stroke in full-term newborns. *Neuropediatrics* 31:202-205.

- Raol YH, Lund IV, Bandyopadhyay S, Zhang G, Roberts DS, Wolfe JH, Russek SJ, Brooks-Kayal AR. (2006) Enhancing GABA(A) receptor alpha 1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal lobe epilepsy. *J Neurosci* 26:11342-11346.
- Rennie JM, Hagmann CF, Robertson NJ, 2008. The baby with a suspected seizure. Neonatal Cerebral Investigation, Second Ed. Cambridge University Press, New York, pp. 92-129.
- Rennie JM, Boylan GB. (2003) Neonatal seizures and their treatment. *Curr Opin Neurol* 16:177-181.
- Rennie JM, Chorley G, Boylan GB, Pressler R, Nguyen Y, Hooper R. (2004) Non-expert use of the cerebral function monitor for neonatal seizure detection. *Arch Dis Child Fetal Neonatal Ed* 89:F37-F40.
- Riazi K, Galic MA, Kuzmiski JB, Ho W, Sharkey KA, Pittman QJ. (2008) Microglial activation and TNFalpha production mediate altered CNS excitability following peripheral inflammation. *Proc Natl Acad Sci U S A* 105:17151-17156.
- Ritter LM, Unis AS, Meador-Woodruff JH. (2001) Ontogeny of ionotropic glutamate receptor expression in human fetal brain. *Brain Res Dev Brain Res* 127:123-133.
- Robertson NJ, Faulkner S, Fleiss B, Bainbridge A, Andorka C, Price D, Powell E, Lecky-Thompson L, Thei L, Chandrasekaran M, Hristova M, Cady EB, Gressens P, Golay X, Raivich G. (2013) Melatonin augments hypothermic neuroprotection in a perinatal asphyxia model. *Brain* 136:90-105.
- Roka A, Melinda KT, Vasarhelyi B, Machay T, Azzopardi D, Szabo M. (2008) Elevated morphine concentrations in neonates treated with morphine and prolonged hypothermia for hypoxic ischemic encephalopathy. *Pediatrics* 121:e844-e849.
- Rousset CI, Baburamani AA, Thornton C, Hagberg H. (2012) Mitochondria and perinatal brain injury. *J Matern Fetal Neonatal Med* 25 Suppl 1:35-38.
- Rutherford MA, Pennock JM, Cowan FM, Dubowitz LM, Hajnal JV, Bydder GM. (1997) Does the brain regenerate after perinatal infarction? *Eur J Paediatr Neurol* 1:13-17.
- Saadeldin IY, Milhem RM, Al-Gazali L, Ali BR. (2013) Novel KCNQ2 mutation in a large Emirati family with benign familial neonatal seizures. *Pediatr Neurol* 48:63-66.
- Saghyan A, LaTorre GN, Keesey R, Sharma A, Mehta V, Rudenko V, Hallas BH, Rafiuddin A, Goldstein B, Friedman LK. (2010) Glutamatergic and morphological alterations associated with early life seizure-induced preconditioning in young rats. *Eur J Neurosci* 32:1897-1911.
- Sampath D, White AM, Raol YH. (2014) Characterization of neonatal seizures in an animal model of hypoxic-ischemic encephalopathy. *Epilepsia* 55:985-993.
- Sanchez RM, Dai W, Levada RE, Lippman JJ, Jensen FE. (2005) AMPA/kainate receptor-mediated downregulation of GABAergic synaptic transmission by calcineurin after seizures in the developing rat brain. *J Neurosci* 25:3442-3451.
- Sanchez RM, Jensen FE. (2001) Maturation aspects of epilepsy mechanisms and consequences for the immature brain. *Epilepsia* 42:577-585.
- Sanchez RM, Koh S, Rio C, Wang C, Lamperti ED, Sharma D, Corfas G, Jensen FE. (2001) Decreased glutamate receptor 2 expression and enhanced epileptogenesis in immature rat hippocampus after perinatal hypoxia-induced seizures. *J Neurosci* 21:8154-8163.
- Sanocka UM, Donnelly DF, Haddad GG. (1988) Cardiovascular and neurophysiologic changes during graded duration of apnea in piglets. *Pediatr Res* 23:402-407.
- Sarnat HB, Sarnat MS. (1976) Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch Neurol* 33:696-705.
- Scharfman HE, McCloskey DP. (2009) Postnatal neurogenesis as a therapeutic target in temporal lobe epilepsy. *Epilepsy Res* 85:150-161.
- Scher MS. (2002) Controversies regarding neonatal seizure recognition. *Epileptic Disord* 4:139-158.
- Scher MS. (2003) Neonatal seizures and brain damage. *Pediatr Neurol* 29:381-390.
- Scher MS, Alvin J, Gaus L, Minnigh B, Painter MJ. (2003) Uncoupling of EEG-clinical neonatal seizures after antiepileptic drug use. *Pediatr Neurol* 28:277-280.
- Scher MS, Hamid MY, Steppe DA, Beggarly ME, Painter MJ. (1993) Ictal and interictal electrographic seizure durations in preterm and term neonates. *Epilepsia* 34:284-288.
- Scher MS, Steppe DA, Beggarly M. (2008) Timing of neonatal seizures and intrapartum obstetrical factors. *J Child Neurol* 23:640-643.

- Schiering IA, de Haan TR, Niermeijer JM, Koelman JH, Majoie CB, Reneman L, Aronica E. (2014) Correlation between clinical and histologic findings in the human neonatal hippocampus after perinatal asphyxia. *J Neuropathol Exp Neurol* 73:324-334.
- Schmitt FC, Buchheim K, Meierkord H, Holtkamp M. (2006) Anticonvulsant properties of hypothermia in experimental status epilepticus. *Neurobiol Dis* 23:689-696.
- Schober A, Sokolova E, Gingrich KJ. (2010) Pentobarbital inhibition of human recombinant $\alpha 1A$ P/Q-type voltage-gated calcium channels involves slow, open channel block. *Br J Pharmacol* 161:365-383.
- Shah DK, Boylan GB, Rennie JM. (2012) Monitoring of seizures in the newborn. *Arch Dis Child Fetal Neonatal Ed* 97:F65-F69.
- Shah DK, de Vries LS, Hellstrom-Westas L, Toet MC, Inder TE. (2008) Amplitude-integrated electroencephalography in the newborn: a valuable tool. *Pediatrics* 122:863-865.
- Shah DK, Wusthoff CJ, Clarke P, Wyatt JS, Ramaiah SM, Dias RJ, Becher JC, Kapellou O, Boardman JP. (2014) Electrographic seizures are associated with brain injury in newborns undergoing therapeutic hypothermia. *Arch Dis Child Fetal Neonatal Ed* 99:F219-F224.
- Shankaran S, Laptook AR, Ehrenkranz RA, Tyson JE, McDonald SA, Donovan EF, Fanaroff AA, Poole WK, Wright LL, Higgins RD, Finer NN, Carlo WA, Duara S, Oh W, Cotten CM, Stevenson DK, Stoll BJ, Lemons JA, Guillet R, Jobe AH. (2005) Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med* 353:1574-1584.
- Shany E, Benzaquen O, Watemberg N. (2007) Comparison of continuous drip of midazolam or lidocaine in the treatment of intractable neonatal seizures. *J Child Neurol* 22:255-259.
- Shellhaas RA. (2015) Continuous long-term electroencephalography: The gold standard for neonatal seizure diagnosis. *Semin Fetal Neonatal Med*.
- Shellhaas RA, Clancy RR. (2007) Characterization of neonatal seizures by conventional EEG and single-channel EEG. *Clin Neurophysiol* 118:2156-2161.
- Shellhaas RA, Ng CM, Dillon CH, Barks JD, Bhatt-Mehta V. (2013) Population pharmacokinetics of phenobarbital in infants with neonatal encephalopathy treated with therapeutic hypothermia. *Pediatr Crit Care Med* 14:194-202.
- Shellhaas RA, Soaita AI, Clancy RR. (2007) Sensitivity of amplitude-integrated electroencephalography for neonatal seizure detection. *Pediatrics* 120:770-777.
- Sheth RD, Buckley DJ, Gutierrez AR, Gingold M, Bodensteiner JB, Penney S. (1996) Midazolam in the treatment of refractory neonatal seizures. *Clin Neuropharmacol* 19:165-170.
- Shuper A, Zalzburg J, Weitz R, Mimouni M. (1991) Jitteriness beyond the neonatal period: a benign pattern of movement in infancy. *J Child Neurol* 6:243-245.
- Sieghart W, Ramerstorfer J, Sarto-Jackson I, Varagic Z, Ernst M. (2012) A novel GABA(A) receptor pharmacology: drugs interacting with the $\alpha(+)$ $\beta(-)$ interface. *Br J Pharmacol* 166:476-485.
- Silverstein FS, Ferriero DM. (2008) Off-label use of antiepileptic drugs for the treatment of neonatal seizures. *Pediatr Neurol* 39:77-79.
- Silverstein FS, Jensen FE. (2007) Neonatal seizures. *Ann Neurol* 62:112-120.
- Simbruner G, Mittal RA, Rohlmann F, Mueche R. (2010) Systemic hypothermia after neonatal encephalopathy: outcomes of neo.nEURO.network RCT. *Pediatrics* 126:e771-e778.
- Singh D, Kumar P, Narang A. (2005) A randomized controlled trial of phenobarbital in neonates with hypoxic ischemic encephalopathy. *J Matern Fetal Neonatal Med* 18:391-395.
- Sirsi D, Nadiminti L, Packard MA, Engel M, Solomon GE. (2007) Apneic seizures: a sign of temporal lobe hemorrhage in full-term neonates. *Pediatr Neurol* 37:366-370.
- Sirsi D, Nangia S, LaMothe J, Kosofsky BE, Solomon GE. (2008) Successful management of refractory neonatal seizures with midazolam. *J Child Neurol* 23:706-709.
- Slaughter L, Egelhoff J, Balmakund T. (2009) Neurologic outcome in neonatal temporal lobe hemorrhagic venous infarcts. *J Child Neurol* 24:1236-1242.
- Smith JR. (1938) The Electroencephalogram During Normal Infancy and Childhood: I. Rhythmic Activities Present in the Neonate and Their Subsequent Development. *The Pedagogical Seminary and Journal of Genetic Psychology* 53:431-453.

- Spitzmiller RE, Phillips T, Meinzen-Derr J, Hoath SB. (2007) Amplitude-integrated EEG is useful in predicting neurodevelopmental outcome in full-term infants with hypoxic-ischemic encephalopathy: a meta-analysis. *J Child Neurol* 22:1069-1078.
- Sreenan C, Bhargava R, Robertson CM. (2000) Cerebral infarction in the term newborn: clinical presentation and long-term outcome. *J Pediatr* 137:351-355.
- Srinivasakumar P, Zempel J, Wallendorf M, Lawrence R, Inder T, Mathur A. (2013) Therapeutic hypothermia in neonatal hypoxic ischemic encephalopathy: electrographic seizures and magnetic resonance imaging evidence of injury. *J Pediatr* 163:465-470.
- Staflstrom CE, Moshe SL, Swann JW, Nehlig A, Jacobs MP, Schwartzkroin PA. (2006) Models of pediatric epilepsies: strategies and opportunities. *Epilepsia* 47:1407-1414.
- Stein V, Hermans-Borgmeyer I, Jentsch TJ, Hubner CA. (2004) Expression of the KCl cotransporter KCC2 parallels neuronal maturation and the emergence of low intracellular chloride. *J Comp Neurol* 468:57-64.
- Stevenson NJ, Korotchikova I, Temko A, Lightbody G, Marnane WP, Boylan GB. (2013) An automated system for grading EEG abnormality in term neonates with hypoxic-ischaemic encephalopathy. *Ann Biomed Eng* 41:775-785.
- Stevenson NJ, O'Toole JM, Korotchikova I, Boylan GB. (2014) Artefact detection in neonatal EEG. *Conf Proc IEEE Eng Med Biol Soc* 2014:926-929.
- Stewart CP, Otsubo H, Ochi A, Sharma R, Hutchison JS, Hahn CD. (2010) Seizure identification in the ICU using quantitative EEG displays. *Neurology* 75:1501-1508.
- Takei Y, Sunohara D, Nishikawa Y, Nagashima C, Tachibana M, Takami T, Miyajima T, Hoshika A. (2005) Effects of rapid rewarming on cerebral nitric oxide production and cerebral hemodynamics after hypothermia therapy for kainic acid-induced seizures in immature rabbits. *Pediatr Int* 47:53-59.
- Takemoto CK, Hodding JH, Kraus DM, 2012. The Pediatric Dosage Handbook, 19 Ed. Lexi-Comp, Inc, Hudson, Ohio.
- Talos DM, Chang M, Kosaras B, Fitzgerald E, Murphy A, Folkerth RD, Jensen FE. (2013) Antiepileptic effects of levetiracetam in a rodent neonatal seizure model. *Pediatr Res* 73:24-30.
- Talos DM, Follett PL, Folkerth RD, Fishman RE, Trachtenberg FL, Volpe JJ, Jensen FE. (2006) Developmental regulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor subunit expression in forebrain and relationship to regional susceptibility to hypoxic/ischemic injury. II. Human cerebral white matter and cortex. *J Comp Neurol* 497:61-77.
- Tan WK, Williams CE, During MJ, Mallard CE, Gunning MI, Gunn AJ, Gluckman PD. (1996) Accumulation of cytotoxins during the development of seizures and edema after hypoxic-ischemic injury in late gestation fetal sheep. *Pediatr Res* 39:791-797.
- Taylor DL, Edwards AD, Mehmet H. (1999) Oxidative metabolism, apoptosis and perinatal brain injury. *Brain Pathol* 9:93-117.
- Tekgul H, Bourgeois BF, Gauvreau K, Bergin AM. (2005) Electroencephalography in neonatal seizures: comparison of a reduced and a full 10/20 montage. *Pediatr Neurol* 32:155-161.
- Temko A, Thomas E, Boylan G, Marnane W, Lightbody G. (2009) An SVM-based system and its performance for detection of seizures in neonates. *Conf Proc IEEE Eng Med Biol Soc* 2009:2643-2646.
- Temko A, Thomas E, Marnane W, Lightbody G, Boylan GB. (2011) Performance assessment for EEG-based neonatal seizure detectors. *Clin Neurophysiol* 122:474-482.
- Tharp BR. (2002) Neonatal seizures and syndromes. *Epilepsia* 43 Suppl 3:2-10.
- Thibeault-Eybalin MP, Lortie A, Carmant L. (2009) Neonatal seizures: do they damage the brain? *Pediatr Neurol* 40:175-180.
- Thompson CM, Puterman AS, Linley LL, Hann FM, van der Elst CW, Molteni CD, Malan AF. (1997) The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. *Acta Paediatr* 86:757-761.
- Thoresen M, Haaland K, Loberg EM, Whitelaw A, Apricena F, Hanko E, Steen PA. (1996) A piglet survival model of posthypoxic encephalopathy. *Pediatr Res* 40:738-748.
- Thoresen M, Hobbs CE, Wood T, Chakkarapani E, Dingley J. (2009) Cooling combined with immediate or delayed xenon inhalation provides equivalent long-term neuroprotection after neonatal hypoxia-ischemia. *J Cereb Blood Flow Metab* 29:707-714.
- Toet MC, Hellstrom-Westas L, Groenendaal F, Eken P, de Vries LS. (1999) Amplitude integrated EEG 3 and 6 hours after birth in full term neonates with hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed* 81:F19-F23.

- Toet MC, Lemmers PM. (2009) Brain monitoring in neonates. *Early Hum Dev* 85:77-84.
- Toet MC, van der MW, de Vries LS, Uiterwaal CS, van Huffelen KC. (2002) Comparison between simultaneously recorded amplitude integrated electroencephalogram (cerebral function monitor) and standard electroencephalogram in neonates. *Pediatrics* 109:772-779.
- Toet MC, van Rooij LG, de Vries LS. (2008) The use of amplitude integrated electroencephalography for assessing neonatal neurologic injury. *Clin Perinatol* 35:665-78, v.
- Tooley JR, Satas S, Porter H, Silver IA, Thoresen M. (2003) Head cooling with mild systemic hypothermia in anesthetized piglets is neuroprotective. *Ann Neurol* 53:65-72.
- Topjian AA, Gutierrez-Colina AM, Sanchez SM, Berg RA, Friess SH, Dlugos DJ, Abend NS. (2013) Electrographic status epilepticus is associated with mortality and worse short-term outcome in critically ill children. *Crit Care Med* 41:215-223.
- Tortorici MA, Kochanek PM, Poloyac SM. (2007) Effects of hypothermia on drug disposition, metabolism, and response: A focus of hypothermia-mediated alterations on the cytochrome P450 enzyme system. *Crit Care Med* 35:2196-2204.
- Toso PA, Gonzalez AJ, Perez ME, Kattan J, Fabres JG, Tapia JL, Gonzalez HS. (2014) Clinical utility of early amplitude integrated EEG in monitoring term newborns at risk of neurological injury. *J Pediatr (Rio J)* 90:143-148.
- Tramonte JJ, Goodkin HP. (2004) Temporal lobe hemorrhage in the full-term neonate presenting as apneic seizures. *J Perinatol* 24:726-729.
- Trauner DA, Chase C, Walker P, Wulfeck B. (1993) Neurologic profiles of infants and children after perinatal stroke. *Pediatr Neurol* 9:383-386.
- Treiman DM. (1995) Electroclinical features of status epilepticus. *J Clin Neurophysiol* 12:343-362.
- Tsuchida TN. (2013) EEG background patterns and prognostication of neonatal encephalopathy in the era of hypothermia. *J Clin Neurophysiol* 30:122-125.
- Tsuchida TN, Wusthoff CJ, Shellhaas RA, Abend NS, Hahn CD, Sullivan JE, Nguyen S, Weinstein S, Scher MS, Riviello JJ, Clancy RR. (2013) American clinical neurophysiology society standardized EEG terminology and categorization for the description of continuous EEG monitoring in neonates: report of the American Clinical Neurophysiology Society critical care monitoring committee. *J Clin Neurophysiol* 30:161-173.
- Turgut M, Uyanikgil Y, Ates U, Baka M, Yurtseven ME. (2006) Pinealectomy stimulates and exogenous melatonin inhibits harmful effects of epileptiform activity during pregnancy in the hippocampus of newborn rats: an immunohistochemical study. *Childs Nerv Syst* 22:481-488.
- Twomey A and Bowden A. National Neonatal Transport Programme Guideline for Cooling on Transport. http://www.nntp.ie/downloads/Cooling_Protocol_for_Publication_19092011.pdf . 2011.
- Tyzio R, Cossart R, Khalilov I, Minlebaev M, Hubner CA, Represa A, Ben-Ari Y, Khazipov R. (2006) Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. *Science* 314:1788-1792.
- Uria-Avellanal C, Robertson NJ. (2014) Na(+)/H(+) exchangers and intracellular pH in perinatal brain injury. *Transl Stroke Res* 5:79-98.
- Uvebrant P. (1988) Hemiplegic cerebral palsy. Aetiology and outcome. *Acta Paediatr Scand Suppl* 345:1-100.
- van den Broek MP, Groenendaal F, Toet MC, van Straaten HL, van Hasselt JG, Huitema AD, de Vries LS, Egberts AC, Rademaker CM. (2012) Pharmacokinetics and clinical efficacy of phenobarbital in asphyxiated newborns treated with hypothermia: a thermopharmacological approach. *Clin Pharmacokinet* 51:671-679.
- van den Broek MP, Huitema AD, van Hasselt JG, Groenendaal F, Toet MC, Egberts TC, de Vries LS, Rademaker CM. (2011) Lidocaine (lignocaine) dosing regimen based upon a population pharmacokinetic model for preterm and term neonates with seizures. *Clin Pharmacokinet* 50:461-469.
- van den Broek MP, Rademaker CM, van Straaten HL, Huitema AD, Toet MC, de Vries LS, Egberts AC, Groenendaal F. (2013) Anticonvulsant treatment of asphyxiated newborns under hypothermia with lidocaine: efficacy, safety and dosing. *Arch Dis Child Fetal Neonatal Ed* 98:F341-F345.
- van der Heide MJ, Roze E, van d, V, Ter Horst HJ, Brouwer OF, Bos AF. (2012) Long-term neurological outcome of term-born children treated with two or more anti-epileptic drugs during the neonatal period. *Early Hum Dev* 88:33-38.
- van der Worp HB, Macleod MR, Kollmar R. (2010) Therapeutic hypothermia for acute ischemic stroke: ready to start large randomized trials? *J Cereb Blood Flow Metab* 30:1079-1093.
- van Rooij LG, de Vries LS, Handryastuti S, Hawani D, Groenendaal F, van Huffelen AC, Toet MC. (2007) Neurodevelopmental outcome in term infants with status epilepticus detected with amplitude-integrated electroencephalography. *Pediatrics* 120:e354-e363.

- van Rooij LG, de Vries LS, van Huffelen AC, Toet MC. (2010a) Additional value of two-channel amplitude integrated EEG recording in full-term infants with unilateral brain injury. *Arch Dis Child Fetal Neonatal Ed* 95:F160-F168.
- van Rooij LG, Hellstrom-Westas L, de Vries LS. (2013a) Treatment of neonatal seizures. *Semin Fetal Neonatal Med* 18:209-215.
- van Rooij LG, Toet MC, van Huffelen AC, Groenendaal F, Laan W, Zecic A, de HT, van S, I, Vrancken S, van WG, van der SJ, Ter HH, Gavilanes D, Laroche S, Naulaers G, de Vries LS. (2010b) Effect of treatment of subclinical neonatal seizures detected with aEEG: randomized, controlled trial. *Pediatrics* 125:e358-e366.
- van Rooij LG, van den Broek MP, Rademaker CM, de Vries LS. (2013b) Clinical management of seizures in newborns : diagnosis and treatment. *Paediatr Drugs* 15:9-18.
- van't Hoff JH, 1884. Etudes de dynamique chimique. Muller and CIE pUBL, Amsterdam.
- Vasiljevic B, Maglajlic-Djukic S, Gojnic M. (2012) The prognostic value of amplitude-integrated electroencephalography in neonates with hypoxic-ischemic encephalopathy. *Vojnosanit Pregl* 69:492-499.
- Venkataraman A, Kingsley PB, Kalina P, Pavlakis SG, Buckwald S, Spinazzola R, Harper RG. (2004) Newborn brain infarction: clinical aspects and magnetic resonance imaging. *CNS Spectr* 9:436-444.
- Vento M, de Vries LS, Alberola A, Blennow M, Steggerda S, Greisen G, Boronat N. (2010) Approach to seizures in the neonatal period: a European perspective. *Acta Paediatr* 99:497-501.
- Vesela K, Honzik T, Hansikova H, Haeuptle MA, Semberova J, Stranak Z, Hennet T, Zeman J. (2009) A new case of ALG8 deficiency (CDG 1h). *J Inherit Metab Dis* 32 Suppl 1.
- Volpe JJ, 2008. Neonatal Seizures. Neurology of the Newborn, Fifth Ed. WB Saunders Company, Philadelphia, PA, pp. 203-244.
- Walsh BH, Low E, Bogue CO, Murray DM, Boylan GB. (2011) Early continuous video electroencephalography in neonatal stroke. *Dev Med Child Neurol* 53:89-92.
- Wang DD, Kriegstein AR. (2011) Blocking early GABA depolarization with bumetanide results in permanent alterations in cortical circuits and sensorimotor gating deficits. *Cereb Cortex* 21:574-587.
- Wang H, Olivero W, Wang D, Lanzino G. (2006) Cold as a therapeutic agent. *Acta Neurochir (Wien)* 148:565-570.
- Wassink G, Barrett RD, Davidson JO, Bennet L, Galinsky R, Dragunow M, Gunn AJ. (2015) Hypothermic neuroprotection is associated with recovery of spectral edge frequency after asphyxia in preterm fetal sheep. *Stroke* 46:585-587.
- Wassink G, Gunn ER, Drury PP, Bennet L, Gunn AJ. (2014) The mechanisms and treatment of asphyxial encephalopathy. *Front Neurosci* 8:40.
- Wasterlain CG, Thompson KW, Suchomelova L, Niquet J. (2010) Brain energy metabolism during experimental neonatal seizures. *Neurochem Res* 35:2193-2198.
- Watanabe K. (2014) Neurophysiological aspects of neonatal seizures. *Brain Dev* 36:363-371.
- Watanabe K, Hara K, Miyazaki S, Hakamada S, Kuroyanagi M. (1982) Apneic seizures in the newborn. *Am J Dis Child* 136:980-984.
- Weiner SP, Painter MJ, Geva D, Guthrie RD, Scher MS. (1991) Neonatal seizures: electroclinical dissociation. *Pediatr Neurol* 7:363-368.
- Whitelaw A. (2012) Periventricular hemorrhage: a problem still today. *Early Hum Dev* 88:965-969.
- Wickstrom R, Hallberg B, Bartocci M. (2013) Differing attitudes toward phenobarbital use in the neonatal period among neonatologists and child neurologists in Sweden. *Eur J Paediatr Neurol* 17:55-63.
- Williams KP, Singh A. (2002) The correlation of seizures in newborn infants with significant acidosis at birth with umbilical artery cord gas values. *Obstet Gynecol* 100:557-560.
- Wirrell EC, Armstrong EA, Osman LD, Yager JY. (2001) Prolonged seizures exacerbate perinatal hypoxic-ischemic brain damage. *Pediatr Res* 50:445-454.
- Wong HK, Liu XB, Matos MF, Chan SF, Perez-Otano I, Boysen M, Cui J, Nakanishi N, Trimmer JS, Jones EG, Lipton SA, Sucher NJ. (2002) Temporal and regional expression of NMDA receptor subunit NR3A in the mammalian brain. *J Comp Neurol* 450:303-317.
- Wong T, Zhang XL, Asl MN, Wu CP, Carlen PL, Zhang L. (2005) Postnatal development of intrinsic GABAergic rhythms in mouse hippocampus. *Neuroscience* 134:107-120.

- Wu J, Ma DL, Ling EA, Tang FR. (2012) Corticotropin releasing factor (CRF) in the hippocampus of the mouse pilocarpine model of status epilepticus. *Neurosci Lett* 512:83-88.
- Wu YW, Lynch JK, Nelson KB. (2005) Perinatal arterial stroke: understanding mechanisms and outcomes. *Semin Neurol* 25:424-434.
- Wusthoff CJ. (2013) Diagnosing neonatal seizures and status epilepticus. *J Clin Neurophysiol* 30:115-121.
- Wusthoff CJ, Dlugos DJ, Gutierrez-Colina A, Wang A, Cook N, Donnelly M, Clancy R, Abend NS. (2011) Electrographic seizures during therapeutic hypothermia for neonatal hypoxic-ischemic encephalopathy. *J Child Neurol* 26:724-728.
- Wusthoff CJ, Shellhaas RA, Clancy RR. (2009) Limitations of single-channel EEG on the forehead for neonatal seizure detection. *J Perinatol* 29:237-242.
- Xu L, Yenari MA, Steinberg GK, Giffard RG. (2002) Mild hypothermia reduces apoptosis of mouse neurons in vitro early in the cascade. *J Cereb Blood Flow Metab* 22:21-28.
- Yager JY, Armstrong EA, Miyashita H, Wirrell EC. (2002) Prolonged neonatal seizures exacerbate hypoxic-ischemic brain damage: correlation with cerebral energy metabolism and excitatory amino acid release. *Dev Neurosci* 24:367-381.
- Yamamoto H, Aihara M, Nijima S, Yamanouchi H. (2007) Treatments with midazolam and lidocaine for status epilepticus in neonates. *Brain Dev* 29:559-564.
- Yang XF, Rothman SM. (2009) Levetiracetam has a time- and stimulation-dependent effect on synaptic transmission. *Seizure* 18:615-619.
- Yap V, Engel M, Takenouchi T, Perlman JM. (2009) Seizures are common in term infants undergoing head cooling. *Pediatr Neurol* 41:327-331.
- Yu JY, Pearl PL. (2013) Metabolic causes of epileptic encephalopathy. *Epilepsy Res Treat* 2013:124934.
- Yue C, Yaari Y. (2004) KCNQ/M channels control spike afterdepolarization and burst generation in hippocampal neurons. *J Neurosci* 24:4614-4624.
- Zangaladze A, Nei M, Liporace JD, Sperling MR. (2008) Characteristics and clinical significance of subclinical seizures. *Epilepsia* 49:2016-2021.
- Zendedel A, Habib P, Dang J, Lammerding L, Hoffmann S, Beyer C, Slowik A. (2015) Omega-3 polyunsaturated fatty acids ameliorate neuroinflammation and mitigate ischemic stroke damage through interactions with astrocytes and microglia. *J Neuroimmunol* 278:200-211.
- Zhang G, Raol YS, Hsu FC, Brooks-Kayal AR. (2004) Long-term alterations in glutamate receptor and transporter expression following early-life seizures are associated with increased seizure susceptibility. *J Neurochem* 88:91-101.

Section 7

Appendix

Parents Information Leaflet



EEG and NIRS testing for Babies

You are being asked to take part in a research study. In order to decide whether or not you want to be a part of this research study, we would like you to know enough about the risks and benefits to make a decision. This is a vital part of the process of fully informed consent. This parent information leaflet provides you with information about the research study currently being undertaken in the neonatal intensive care unit of the Cork University Maternity Hospital. Once you understand the study, you will be asked to sign this form if you wish to take part. This information leaflet is for you to keep.

1. What is this study all about?

The doctors in Cork University Maternity Hospital have experience in studying brain waves in sick newborns. Your baby may have unusual movements, is sick and may be at risk of seizures often called *fits or convulsions*. To confirm whether your baby is having seizures or not, we would like to do a test called *EEG* (*electroencephalogram*). To know whether the oxygen supply to your baby's brain is affected or not we would like to do a test called *NIRS* (*near infrared spectrometry*).

2. What is a fit, convulsion or seizure?

Fits or convulsions are also called seizures. They are due to excessive electrical activity in the brain.

3. Why does my baby need an EEG?

Your baby's behaviour suggests that he/she may be having seizures. The EEG test is carried out to help confirm that these events are actually caused by seizures.

4. What is an EEG?

An EEG is a specialized test that picks up tiny electrical signals from your baby's brain. A series of soft discs will be placed on your baby's head with a special paste. These discs pick up the brain's electrical activity and are connected to a machine by short wires, which record this activity (the wires do not give out electrical signals). At the same time, your baby will be videoed to monitor your baby's movements which can be important and help us understand the brain activity better. Once the test is complete the discs will be removed. There may be some residue of the soft paste on your baby's head that will wash off with warm water.

5. What is NIRS?

NIRS is a test that picks up the oxygen level in your baby's brain. Like the EEG soft discs, the NIRS discs will also be applied on your baby's head and will be kept in place along with the EEG test for the same length of time and will also be removed the same way.

6. How long will the whole study take place on my baby?

The EEG and NIRS discs take about 30 minutes to apply and after this, they will be left in place for up to 3 days while your baby is being monitored.

7. Will there be any side effects from performing the EEG and NIRS on my baby?

There are no side effects from the EEG and NIRS to your baby.

8. Will my baby feel any pain when the EEG and NIRS monitors are on my baby's head?

The EEG and NIRS are a safe and painless procedure. We can assure you that we will apply the soft recording discs carefully and slowly to ensure that your baby is disturbed as little as possible.

9. Will I be allowed to see my baby during this study?

Of course. We will encourage you to see your baby as much as you can while your baby is in the Neonatal Intensive Care Unit.

10. How soon will I know the result of the tests?

The results of the tests may not be available until the tests are completed over a number of days. As soon as the doctors have results, they will discuss them with you.

11. How can it help my baby?

When the doctors have the results of the tests, it may help them to provide you with the diagnosis of your baby's condition and may also help with treatment of this condition. The doctor looking after your baby will explain this to you in greater details if your baby requires treatment.

12. Are EEG and NIRS widely used in other hospitals?

It is used in Neonatal Intensive Care Units worldwide and we are very fortunate to have this monitoring available in Cork University Maternity Hospital. EEG and NIRS are also commonly used in older children and adults.

13. Will my baby require any other procedures during this study?

We wish to examine your baby and ask you a few simple questions about the pregnancy and delivery. To conduct this research thoroughly, we will also require information from the medical notes of both mother and baby. This information and the EEG data that we record will help us to develop an automated computerized system for monitoring newborn babies at risk of seizures. Further investigations such as blood test, lumbar punctures and radiographical imaging may take place but only at the discretion of your attending doctor and these are not directed by the study.

14. Will my identity as well as my baby's identity be revealed?

No. All information will be stored securely and will be treated with the strictest confidence. We would like your permission to show this information to other medical professionals for research and teaching purposes on occasion but will not reveal any identifying details about yourself or your baby.

15. Do I have to take part in this study?

You do not have to take part in this study if you do not want to. If you decide to take part, you may withdraw at any time without having to give a reason. Your decision on whether to take part or not, will not affect the care and management

of your baby in any way. We understand that this is a very difficult time for you and will do our best to provide you with all the information that you need at any time. We also wish to emphasize that your baby may need to have these tests anyway as part of the routine clinical care however it will be for a much shorter time. In the past, parents have not found our studies intrusive and in fact have been very reassured by the added benefit of this continuous monitoring.

If you decide to take part, you will be given a copy of this information sheet to keep and be asked to sign a consent form, a copy of which you will also be given to keep.

16. Will my baby be followed up in the future?

After your baby has gone home, we would like to see how your baby is doing for neurodevelopmental milestone and outcome. Therefore, we will contact you within 18 months and 2 years to see how your baby is getting on.

17. Who should I contact if I have more questions about this study?

Do not hesitate to speak to the Consultant looking after your baby or any of the research doctors if you need more information about this study. We will be happy to answer any questions that you may have.

After you have read this information and once you have fully understood the procedures, the doctors will ask you to sign a consent form. The doctors will discuss any issues you may have about the test.

(Copy for Investigator and a copy for Parent)

CONSENT BY PARENTS FOR PARTICIPATION IN STUDY

Protocol Number: _____ Baby's Addressogram: _____

Title of Protocol: Seizure Detection for Babies

Doctor(s) Directing Research:

Prof Anthony Ryan, Prof Geraldine Boylan, Dr Deirdre Murray, Dr Brendan Murphy, Dr Peter Filan, Dr Gene Dempsey, Dr Liam O'Connell, Dr Irina Korotchikova, Dr Brian Walsh, Dr Niamh Lynch, Dr Evonne Low (Mobile number).

AGREEMENT TO CONSENT

The research project and the treatment procedures associated with it have been fully explained to me. All experimental procedures have been identified and no guarantee has been given about the possible results. I have had the opportunity to ask questions concerning any and all aspects of the project and any procedures involved. I am aware that participation is voluntary and that I may withdraw my consent at any time. I am aware that my decision not to participate or to withdraw will not restrict my access to health care services normally available to me. Confidentiality of records concerning my involvement in this project will be maintained in an appropriate manner. When required by law, the records of this research may be reviewed by government agencies and sponsors of the research.

I understand that the sponsors and investigators have such insurance as is required by law in the event of injury resulting from this research.

I, the undersigned, hereby consent my baby to participate as a subject in the above described project conducted at the Cork Teaching Hospitals. I have received a copy of this consent form for my records. I understand that if I have any questions concerning this research, I can contact the doctor(s) listed above. If I have further queries concerning my rights in connection with the research, I can contact the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Lancaster Hall, 6 Little Hanover Street, Cork.

After reading the entire consent form, if you have no further questions about giving consent, please sign where indicated.

Doctor's name: _____ Parent's/ Guardian's signature: _____

Witness's name: _____ Date: _____ Time: _____ (0 to 24 hour)